SOMA AND GERM[1]

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THE conception that germ-cells are derived directly from the fertilized ovum and not from the tissues of the body and that they persist as distinctive elements side by side with the body material, dates apparently from the studies of Nussbaum on Rana, Triton and Salamandra, published in 1880. The tremendous impetus given by Weismann to this idea of the uniqueness and independence of the germ, in his challenge to the doctrine of inheritance of somatic acquirements, has made it the prevailing view to-day, and in the form of the familiar theory of germinal continuity it constitutes the very warp and woof of modern genetics. Since the time of its inception one important discovery after another in the fields of embryology, cytology and genetics has helped to build up the imposing body of facts which in the main substantiates it.

No biologist to-day, in the light of available evidence, would question the conception that the materials of the germ-cells have been derived, in the main at least, from the oosperm by a process of cell-division, for this is the obvious explanation of how hereditary traits already established are handed on.

Because a theory of germinal continuity accounts admirably for the transmission of established genes, how-

[1] Address of the retiring president of the American Society of Zoologists, Washington, D. C., December 31, 1924.

ever, it does not follow necessarily that it also accounts adequately for the acquisition of new genes or for the modification or loss of old ones. Manifestly the cash underlying a bank account can be transmitted from bank to bank indefinitely, but it takes some outside intervention to credit us with two dollars on the ledger where but one has stood before. In the end, in any such hand-and-glove relationship as exists between organism and environment, it would seem that directly or indirectly environment must play an important part in initiating, or at least in conditioning, changes.

We may, perhaps, with some justification, look upon anything that environment brings out as in a sense hereditary—and one of our greatest biologists has apparently taken this stand—but if we accept this broad interpretation as the unqualified truth, then any argument about inheritance of somatic acquirements is pointless, since the very fact that it can be called forth or "acquired" through some specific influence of the environment shows that the "makings" were already present in the germ-plasm and that it only required a special environmental condition to call the character forth. In other words, the thing to be accomplished is already accomplished before it *is* accomplished. A very enigmatical situation, to say the least, I think you will agree. On this basis, then, unless biologists are unique creatures who dwell habitually in a topsy-turvy land, there must be two kinds of heredity; for when we demand evidence of the *inheritance* of such an acquirement we say it must not only be capable of being brought out in this unusual way but that also it must so change the genes of the germ-cells that they will produce the character again in later generations in an environment lacking the original evocative peculiarity.

Perhaps this is a foolish demand; however, it is the one usually made for proof of inheritance of a somatic acquirement. I am not solving the riddle, merely voicing it. On the other hand, is not stating the problem of heredity this way on about the same shadowy basis as

affirming that the potentia of the oceans, continents and topographical features of the world to-day were present in the original nebula which preceded our solar system or that man lived potentially in some primitive protozoa-like creature? While in a sense this may be true I do not believe that the most optimistic of geneticists would expect to find the genes of man's present manifold attributes in such primitive protoplasmic creatures.

What seems to have happened in the evolution of living things is that such originally indefinite "might-bes" have become the present "ares." In other words, of the many possible outcomes inherent in the very physical and chemical nature of the vague initial life-substance, some have been realized. Furthermore, in the organisms of to-day this realization has progressed from the realm of indefinite possibility to a more or less specific certainty through the origination in cells of representative packets which we call genes. Through the action of these, subject to the existing environment, the characteristics of present-day forms are achieved. In other words, no matter how devious the path of procedure or how tangled the skein of chemical relationships, there is a certain determinate correspondence which did not exist in the primitive ancestral protoplasm, between special elements in the germ and the characteristics of the body as they are expressed to-day.

If this is untrue, then either there is no problem of evolution or it is wholly different from what it is commonly conceived to be. If it is true, then the great key problem of evolution—that of the origin of inheritable variations—is this very one of how the genes which exist in germ-cells to-day have been gradually incorporated in the erstwhile primitive protoplasm. This accumulation, which most of us take for granted, has not been a mere hit-or-miss affair, moreover, but an orderly, sequential process. Each new acquisition has been possible largely because of some earlier acquisition. In an organism the past history of a part is an important factor in its pres-

ent expression. Where once reigned possibilities which might be epigenetically realized, there now exists a new, more specifically determinative mechanism which persists as the established constitution of the germ.

Have the genes in question come into existence through the intermediation of the body, and if so was the influence general or specific? Are they attributable directly or indirectly to the external physical environment? Have they arisen as the result of internal constitutional changes which bear no direct relation to the body? Or are they the outcome of now one, now another, or of combinations of these agencies? These are the fundamental questions which confront us and which are likely long to confront us.

When one leaves the cemented highways of text-books and popular treatises on heredity, along which he glides from generalization to generalization, and seeks to penetrate the thicket of results from actual study of embryogenesis in individual organisms, he is likely to get a considerably modified conception of the separateness of the germ-cell from the soma. He finds that instead of a convincing demonstration of universal direct descent of the germ-cells from the original oosperm, actual visible proof of this has been brought forward in a very limited number of cases. Of these the highly specialized pedogenetic fly, Miastor, is one of the most outstanding examples.

Among plants and the lower animals, of course, it has long been recognized that it is futile to try to discriminate sharply between germplasm and somatoplasm because the process of propagation by budding, the facts of the regeneration of removed parts and similar phenomena abundantly attest the reproductive capacity of the somatic cells.

While inferentially, in the fairly limited number of cases in the vertebrates which have been investigated to determine the origin of the germ-cells, these cells are set apart from the somatic cells in early embryogeny, the evidence is by no means indisputable. The best that can

be said is that in most of these cases the primordial germ-cells are recognizable early in the embryo, and that whether they come directly from the original oosperm or not, they arise at least from embryonic cells which have not differentiated into adult somatic tissues. But whether they arise from endoderm, mesoderm, mesenchyme or peritoneum is still in dispute.

Of vertebrates, the amphibians seem to have been worked upon most extensively. To realize how conflicting the testimony is one has only to read Professor George T. Hargitt's recent paper[2] on germ-cell origin in the newt, *Diemyctylus viridescens,* in which he reviews and discusses this field.

But whatever the origin of these primordial germ-cells may be, the evidence is by no means complete that they become the functional germ-cells. Various observers agree that in the amphibia there is extensive degeneration of the primordial germ-cells and that the origin of the functional germ-cells which succeed them is, to say the least, uncertain. Some would derive them from primordial germ-cells which had persisted in spite of the general disintegration of their kind, while others think the evidence shows derivation from adjacent tissues. Gatenby,[3] for instance, in his 1916 study finds that although the primordial germ-cells of Rana temporaria are entodermal in origin, they are of minor significance, for the greater number of definite germ-cells arise from the peritoneum, and he expresses the opinion that any peritoneal cell may become a germ-cell. Swingle[4] in his 1921 study finds that no germ-cells are detectable in larvae of the bullfrog (*Rana catesbeiana*) under 7 mm in length. At this stage they arise from entodermal tissue dorsal to the archenteron and keep multiplying until the larvae reach a size of 40 mm. The primordial germ-cells then degenerate for the most part, and a second generation is produced

[2] *Jour. Morph.,* September, 1924.
[3] *Quart. Jour. Mic. Sci.,* 1916.
[4] *Jour. Exp. Zool.*

which becomes the functional germ-cells of the adult. This second generation, he thinks, comes from a few undegenerated survivors of the original lot.

In the light of all the earlier papers on the source of the functional germ-cells in the embryos of various amphibia and of the successive supplies of spermatozoa in the testicular cysts of urodeles, Hargitt[5] has made a study of the origin of the spermatogonia of adult newts. He finds that in Diemyctylus when the cysts are once emptied they are generally without residual cells and "that there is abundant evidence of the formation of spermatogonia from the epithelial cells of the collecting ducts such as McGregor found in Amphiuma." He even finds isolated nests of germ-cells occasionally in the mesenteries and peritoneum outside the testes and he regards them as probably modified peritoneal cells.

To meet this situation, one way of cutting the Gordian knot, if we still find it necessary or expedient to talk about germplasm, is to follow the lead of those who have come to regard the germplasm as essentially the chromatin material of the cells. Since, through mitosis, this has descended from the original oosperm, obviously all cells have it, though ordinarily only the cells specialized as reproductive cells pass it on in the form of a new generation.

The production of double monsters, of identical twins or of several individuals from one original oosperm, as in the armadillo, indeed, lends further color to the view that all the descendants of the fertilized ovum, germinal and somatic cells alike, retain the hereditary potencies that existed in the original zygote. It has been shown repeatedly through experimental embryology and experimental morphology that in all probability a particular cell takes on the characteristics of a specific tissue, not because it differs constitutionally from other cells, but because of the differential nature of the stimuli to which it has been earlier exposed, or to which it is subjected as

5 *Jour. Morph.*, September, 1924.

a result of its special location in the developing organism. The various phenomena which make up the field of experimental morphology to-day—heteromorphosis, regeneration and regulation, experimentally produced changes of axial gradients, dedifferentiation and redifferentiation, metaplasia and compensatory substitutions—all alike bear witness to the fact that the same initial cellular materials may yield very different end-products under different conditions; that many, possibly all, cells retain the fundamental constitutional mechanisms of the original oosperm.

Since this is true, then the argument commonly advanced against the possibility of a change in such a structure as brain or muscle being specifically paralleled in any degree in the germ, because the germ is a cell which does not possess a brain or a muscle, is largely beside the point. For brain or muscle is, after all, made up of cells each possessing hereditary potencies similar to those of the germ-cells. Any influence therefore that can induce changes in the fundamental constitution of a brain cell or a muscle cell, particularly if it were applied through the circulation media of the body, might conceivably affect the corresponding elements of the germ-cell. If an injury to or a change in a given tissue, for example, induces serological effects which react on the tissue itself, it seems permissible, without doing violence to the possibilities of the case, to suppose that the germ-cells might be also specifically affected.

Two obvious possible sources of influence from the blood stream, apart from the customary nutritive flux, are the internal secretions and the antibodies of various types which may come to occupy the blood.

The whole field of endocrinology is so much in the foreground at present that it is superfluous to enter into lengthy citations. The pronounced effects which may result in even a distant part of the body from the under- or over-secretions of some endocrine gland are known to you all. Of these none is more interesting to the biologist

perhaps than the striking modifications of secondary sexual characters that have been brought about in the various castration, transplantation or injection experiments which are being reported in our present literature.

As an example of how far-reaching such experimental alterations may become I may cite the experiments of my associate, Dr. Hisaw, who in the pocket-gopher (*Geomys bursarius*), through the injection of ovarian material into males, induced in them absorption of a bar-like part of the pubic symphysis. This absorption occurs regularly in young females as the breeding season approaches, but the pubis of the male normally persists throughout life.

Concerning the possibility of antibodies affecting the germ-cells I have already spoken several times before this society, and I shall not weary you by repeating the details of the experiments which have led me to believe that they may be a source of germinal influence. With your indulgence I shall merely recall a few of the outstanding facts.

Two wholly separate lines of defective-eyed rabbits were secured by immunizing fowls against rabbit-lens and then injecting the blood-serum of such fowls into pregnant does. A third unrelated strain was secured by directly injecting rabbit-lens into the pregnant mother. In all cases the defects first appeared in the young of such treated mothers, not in the mothers themselves. As reported in our papers on the subject we had many more negative than positive results. Once the defects were engendered they proved to be hereditary. In the oldest strain they have been transmitted through nine generations. The defects included not only opaque or otherwise abnormal lenses, but also occasionally, microphthalmia, detached retina and other noticeable malformations. Of over 100 of the eyes which have been carefully mounted and studied by an ophthalmologist, Dr. F. A. Davis, every one showed some degree of coloboma. It is not unreasonable to believe, however, though it can not be proved, that the defects may all be traceable to initial lens-disorder

which induced aberrancies or suppressions of normal eye-development. In some instances Dr. Davis has found that certain individuals of our defective-eyed strains which we had recorded as normal, were really abnormal in that the lenses were large and spherical instead of somewhat flattened as they are when under normal tension in normal eyes. One of my research assistants, Mrs. Mabel G. Boyden (paper in press), has shown by means of careful tonometric methods, furthermore, that in some individuals of the defective-eyed strains, even when defects are not visible, there may be abnormal intra-ocular pressure.

We have also secured lens defects on two separate occasions in the young of guinea pigs by treating the pregnant mothers, one with pulped swine lens, the other with pulped rabbit-lens, but inasmuch as these young died before reproducing we can say nothing regarding the inheritability of the condition in guinea pigs.

Recently we have gone about our experimentation in a different manner. We found that when its lens is injured a normal rabbit will develop lens antibodies in its own blood-serum. In such operations the eye is treated with a local anesthetic, then pierced with a needle by means of which the lens is more or less broken up. The procedure is the same as that commonly followed by ophthalmologists in cases of children with congenital cataract.

The serum of all rabbits used in such experiments was first tested for rabbit-lens precipitins and without exception found negative in all dilutions commonly employed in making precipitin tests. In from 7 to 10 days after the needling operation the serum of each rabbit was again tested for lens precipitins. Out of 14 rabbits thus treated 4 gave negative, 2 doubtful and 8 positive results. The lowest positive titer was a dilution of 1 to 40, the highest, 1 to 1,280. Some of those which were negative after the first operation, however, gave positive precipitin reactions after a second operation.

Inasmuch as an individual can evidently develop antibodies against its own injured tissues the question arose

as to whether or not transmitted defects, similar to those of our earlier experiments, could be secured by this method. With this in mind we have at present a number of individuals under experimentation. Our experiments are of too short duration to be reported in any detail yet beyond relating that two different females (female No. 10 and female No. 26) with needled lenses have produced young with opaque lenses. The parents were normal animals bred in our own laboratory from stock we are testing out by inbreeding for genetical purposes. The first litter (nine in number) of female No. 10 by male No. N1A5 were all normal-eyed, notwithstanding the fact that the parents had had their eyes needled before the mating. The eyes of each parent were reneedled before the second mating. There were eight young in the second litter, every one of which had white chalky-looking lenses or lenses with white patches. Unfortunately these young have all died. Their eyes have been mounted, however, by Dr. Davis, and unlike the affected eyes of the earlier defective-eyed strains not one of them shows coloboma. The defect is more exclusively a lens defect.

Female 26, after the first mating to male N10A2, likewise gave birth to a normal-eyed litter, and similarly her eyes were reneedled. The eyes of the male were not needled for either mating. A second litter of four was born to 26, but she did not care for them well and only two lived to open their eyes. The lenses of these two, however, were opaque. So far five other females similarly operated upon have borne young, but none of these young have shown eye-defects.

Whether or not this latter method of experimentation will yield heritable defects can not be determined, of course, until the experiments are carried further. In any event, they give us just that much additional evidence that unborn young are open to serological influences from the mother. Furthermore, the operation is more nearly the kind of thing that might happen in nature than is the introduction of an alien serum into a pregnant mother.

Our published results, or more particularly our tentative conclusions, have not gone unchallenged. Most of the criticism has been justifiable, intelligent and helpful. Unfortunately—for the experimenters at least—the work is of such a nature that it has, or seems to have, bearings on a favorite theme for debate, upon which every argumentative person, from Pullman porter to professor of Greek, apparently claims sovereign right to an authoritative opinion; namely, the inheritance of somatic acquirements, or, as it is usually ambiguously stated, the inheritance of acquired characters. Needless to say we can not sponsor some of the uses—or better, misuses—to which our results have been turned in this connection. I raise this point simply because at different times I have seen such statements of what allegedly we "claim," or what we are "trying to prove," that I feel justified in offering a word of protest.

As a matter of fact, inspection of our published papers will show that we "claim" nothing beyond the right to make a few tentative suggestions as to the possible significance of our results. In the main we have been, and shall remain, content to record the facts as we find them. As to what we are "trying to prove" it should be needless for an experimental scientist to have to explain that he is not trying "to prove" anything, but merely attempting to find the facts in the case. To do this most expeditiously, to be sure, he may proceed by formulating various working hypotheses.

Let us now turn briefly to some of the more constructive criticism.

Professor Stockard[6] reiterates the fact that I myself have pointed out in practically all my papers on the subject that because of the unusual susceptibility of a developing eye to almost any kind of adverse influence, one might well question the specificity of the origin of a fetal eye-anomaly. From the very beginning of our work I have been inclined strongly to interpret the condition as

6 AM. NAT., LVIII, 23-35, 1924.

the result of some general rather than of a specific influence, but the reason I can not accept this explanation as valid is the lack of evidence. The evidence from our controls all points toward specificity. We have had well over a thousand young born from mothers which had been subjected to just as violent or even more violent serological treatments in these and other experiments and never in a single instance have such young had defective eyes. Eye abnormalities have occurred only when a foreign serum which contained lens antibodies was used, or when the mother's own serum carried antibodies developed against lens injected directly or against her own lenses following their injury. Speaking of our experiments, Professor Stockard regards "the doses used as slightly less than a fatal amount," and he seems to think that in some way the foreign serum penetrates the placenta and is toxic to the young. He asserts, for instance, that we have "interpreted the result as a specific response of the germplasm to the lens antigen," when as a matter of fact we interpreted our results as probably a specific response to a lens *antibody*—a very different process. In our experience, although we have repeatedly tried, we have never, with one doubtful exception, succeeded in getting an antigen to penetrate the placenta. We have established, however, that at least certain classes of antibodies do penetrate the placenta. We have a paper in press at present on this aspect of the work.

I would not, however, take the position that antigen may not, as the result of accident, occasionally enter the fetal blood-stream. We have had a student, H. W. Mossman (paper in press), working for three or four years, by double-injection and histological methods, on this very point of the finer structural relations between the fetal and the maternal blood-vessels in the rabbit placenta. As a result of his studies and those of earlier investigators it is clear that after the twelfth day of pregnancy there are areas in which the maternal and the fetal blood

streams are separated by only a single thin layer of fetal epithelium, and from the 22nd day on these areas are extensive. It seems not improbable, therefore, that as a result of the violent struggling a rabbit sometimes does when improperly picked up, this thin epithelium might be ruptured in places in the pregnant doe and thus permit more or less intermingling of the two blood streams. However, while apparently this might easily occur, I have no evidence that it does.

Finally, as regards specificity of effects, it might be pointed out that because a certain type of defect may result from various general adverse agents or conditions, it does not follow that it may not also result from a specific influence.

Huxley and Carr-Saunders,[7] in a paper entitled "Absence of prenatal effects of lens antibodies in rabbits," report the results of experiments in which they have rather closely though not fully paralleled our technique. Their results were negative with the possible exception of one very doubtful case. Twenty-nine does were treated with fowl-serum which had been immunized against rabbit-lens, ox-lens or rabbit- and ox-lens. Twenty-one of these bore fifty-seven young, all normal, with the possible exception already mentioned. Passing over other differences in technique I will only point out that they appear to have used the lenses of adult or nearly mature animals as antigen, while in the main we used the lenses of very young rabbits. Older lenses are tough and are even partially pulped only with difficulty, whereas the lenses of young rabbits go into almost complete solution or suspension. There may have been quantitative or even qualitative differences involved, therefore, in the original antigen.

Incidentally, it may be pointed out that some of their dosages were far in excess of the ones we used, so that if a general poisonous effect of a foreign serum were responsible for the production of eye-defects in unborn

[7] *Brit. Jour. Exp. Biol.*, I, 2; Jan., 1924.

young, as Stockard believes, then some of these might
well have been expected to have shown such defects.

Again, Huxley and Carr-Saunders injected three does
directly with rabbit-lens and six with ox-lens. Six of
these died. The other three bore 17 young, all with nor-
mal eyes.

Negative results always have a certain value, of course,
in experimental work where the conditions of the experi-
ments are fairly parallel. If negative results alone, how-
ever, are the significant thing, then in the aggregate we
can show many more than do Huxley and Carr-Saunders,
for we have had far more failures than successes. All of
which shows that the condition, in its visible form at
least, is not an easy one to induce. Many complex factors
are involved in such experiments and it is extremely dif-
ficult or even impossible, to secure similar conditions in
all respects. The question seems to resolve itself into—
not how much chaff you get—but is there any wheat? We
have secured positive results with sufficient frequency to
make it reasonably sure that the condition is not mere
accidental coincidence. We have checked adequately
against the possibility that it might be the uncovering of
a Mendelian recessive which had in some mysterious way
become incorporated independently in our several dis-
tinct strains of experimental rabbits. For even suppos-
ing that the improbable happened—that in our first ex-
periments we just chanced to hit upon two individuals
which were carrying recessive eye-defects, ready to be
revealed in their offspring independently of our treat-
ments, it is still more improbable that a similar defect
would happen by chance in our unrelated 16A1 line; and
the theory of chance, it seems to me, becomes wholly
untenable when we attempt to carry it over to still a third
unrelated strain, the 84 line, to say nothing of our later
production of congenital cataract by lens-injury in
parents, in still two other wholly unrelated varieties of
rabbits. So that whatever may be the final explanation
of just what has taken place in the germplasm, we feel

assured that the results are in some way the direct out-
come of our experimental treatment.

It has been objected that we used albino rabbits and
that their eyes are already defective to the extent at least
of being devoid of pigment. In answer to this I may say
that neither of the last two does were albinos, in the
young of which opaque lenses have been induced, nor was
one of the guinea pigs. The defect once engendered,
furthermore, can be crossed into pigmented as readily
as into albino strains.

The fact that defective-eyed strains of rats, mice and
guinea pigs have appeared from time to time in various
laboratory stocks has also been argued as an objection.
Until it can be shown how such so-called "spontaneous"
anomalies originate, however, I fail to see just how they
constitute a valid objection. They certainly do not arise
uncaused.

In a recent paper[8] Little and Bagg raised the objection
that an outcross of an abnormal male with an unrelated
normal female, followed by extraction of the defect
through the breeding of such progeny to the same or to
another abnormal male, does not prove inheritance, since,
to quote them, "the direct use of an abnormal male in-
volves the possibility that transmission of an agent pro-
ducing an abnormality might be brought about by the
seminal fluid or other material transferred with this
sperm at copulation," and they imply that our pedigrees
are inconclusive in this respect. They go on to say: "The
most satisfactory type of test is an outcross of a normal
male with unrelated normal females followed by brother-
to-sister inbreeding of the normal progeny from this
cross." However, if they will but examine our Fig. 4,
on page 463, *Journal of Experimental Zoology,* for Jan-
uary, 1924, they will find that we have done even better
than this. A defective-eyed male (3A1) was mated to a
normal unrelated female (11). Two of the normal-eyed
daughters (53A2 and 53A1) were mated respectively to

[8] *Jour. Exp. Biol.,* 41, 1; Nov., 1924.

normal unrelated males (25 and 55A1), and a normal-eyed male offspring (90A2) of one of these matings (25 × 53A2) was finally bred to a normal-eyed female offspring (91A4) of the other mating (53A1 × 55A1) with the result that in the ensuing litter of six two defective-eyed individuals—one male and one female—reappeared. In other words, the defect introduced through male 3A1 was made to reappear in his greatgrandchildren after it had passed in a recessive condition through two normal-eyed females in one generation and a normal-eyed male and normal-eyed female in the next generation. Moreover, pigmentation was introduced into the stock through male 25, and these great-grandchildren of 3A1 not only show marked eye-defects but show them in pigmented eyes. In our published papers, inasmuch as we were concerned primarily with other than genetical data, we have shown only a few of our pedigree charts. Upon looking over our records, however, I find still other cases which fulfill the requirements specified by Little and Bagg.

When one takes into account all the facts, therefore, there can be no reasonable doubt, I think, of the true hereditary nature of the anomaly, nor that it has been engendered in some way by means of our serum-treatment. As already pointed out, in spite of one's general inclination from knowledge of the field of experimental embryology to attribute the initial defect to a general poisonous or inhibitive agent, all our own results checked by over a thousand controls point to it as specific rather than general. We have never obtained the defects in question except in association with a serum carrying specific antibody. But even should the defect have originated from a general rather than a specific cause, the germinal basis must have become specific since the anomaly reappears generation after generation without any recognizable accompanying malformations of other parts of the body. In my opinion, however, it is not without the range of possibility that an eye-defect, however engendered in the first place, might lead to the develop-

ment of antibodies which could inaugurate specific
changes in the germinal correlatives of the somatic parts
affected.

Are our results an example of the inheritance of a
somatic acquirement? We have never maintained that
they are. It is not yet clear whether the eye of the fetus
is first changed and the condition is then conveyed from
it to the germ-cells of this individual, or whether the eye
and the germ of the fetus are influenced separately by
the antibodies. Obviously only the first occurrence could
be interpreted as the inheritance of a somatic change;
the second would be an instance of parallel induction.
Strictly interpreted, even in the defective-eyed young of
mothers with needled lenses, supposing the condition
proves inheritable, the same two alternatives confront us.
Until adequate outbreeding experiments with needle-eyed
males have been made the question must remain unan-
swered. Such experiments are in progress.

Admitting, as I think we shall have to, that the distinc-
tion between soma and germ has often been overempha-
sized, we shall have to recognize that in germ-cells we are
dealing with protoplasmic complexes not essentially dif-
ferent from those of the somatic cells. And it follows
that we can not look on germ-cells any more than on
somatic cells as insusceptible to the ebb and flow of the
chemical or other physiological influences within the body.
Should the germplasm prove to be thus open to modifi-
cation, this does not, of course, carry with it the impli-
cation that all changes in it are initiated in this way, or
that the main current of inheritance is due to somatic
influences. Even granting the possibility of somatic in-
terference there is no reason for supposing that genes
once established would not persist indefinitely in the germ
unless the somatic changes in question have some direct
bearing upon them.

Apart from specific influences, however, recognition of
the close physiological dependence of the germ on the
rest of the body makes it easier for us to conceive of how
many influences might be brought to bear on the germ-

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cells which might lead to constitutional germinal changes not specifically related to the impinging cause. Such a conception would seem to open the way more clearly to a rational explanation of the origin of variations. Even Weismann, in his theory of germinal selection, since the food of his hypothetical components must come from without, had virtually to admit that the inception of germinal variation as he conceived it was environmental in origin.

The fact is that biologists have never yet hit upon an explanation of the marvelous adaptedness of organisms to their environment which does not put a tremendous strain upon our credulity. And when we view it with unprejudiced eye it seems to me that the theory which would attribute adaptation to the mere accumulation of such chance variations as happen to be favorable is the most incredible of all. Time and again in the past, according to paleontologists, whenever new possibilities for existence occurred, forms of life admirably adapted to those conditions have come to occupy the new habitat. In some way the environment has molded these new inhabitants to its bounds, and it takes more faith than I personally possess to believe that it has all been done by the negative method of killing off, generation after generation, the non-conformists—those in which the happy accidents, or rather the innumerable interrelated series of accidents, has not occurred. It seems more within the bounds of credence to suppose that biologists have failed as yet to discover some more direct principle of adaptation whereby living organisms are fitted to their environment. And since in the higher animals, at least, we find abundant evidence of compensatory and other adaptive somatic responses initiated through the agency of the serological or humoral mechanisms of the organism, it would seem well worth while to seek in the same direction for light regarding the possible existence of some form of adaptive variation. For the germ-cells no less than their somatic brethren are open to the surge and sweep of the circulating fluids of the body.

SEX DETERMINATION AND SEX DIFFERENTIATION[1]

SEX DETERMINATION AND SEX DIFFERENTIA-
TION IN THE HIGHER PLANTS

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WHEN Mendelian heredity became definitely known, it was quite natural, of course, that the question as to the Mendelian nature of sexuality should present itself, and since it was well known that in the higher animals and some diecious plants the ratio between the two sexes is about equal, the hypothesis of homozygous and hetero-zygous constitutions was soon formulated. The basis for such a hypothesis seemed quite secure because of the known fact of unequal pairs of allosomes in the higher animals. Thus the hypothesis of sex-determining factors or sex-determining chromosomes has become to many a settled conviction. But to a botanist who has to deal with sexuality as it is actually manifested in the vast majority of gametophytes and heterosporous sporophytes, the phenomena of male and female sex, both as to determina-tion and differentiation, appear to lie mostly beyond the processes of chromosome and factor shiftings and take on a physiological aspect for the simple reason that in the usual type of gametophyte and sporophyte there is no sex determination either at the time of fertilization or of reduction. It is evident to any one acquainted with the animals, also, that the problem does not fit into any such simple strait-jacket as a Mendelian formula would

[1] Symposium on Sex Determination and Sex Differentiation presented be-fore the meeting of the American Society of Zoologists at Washington. D. C., December 31, 1924.

require. The matter presents itself in sharp contrast to ordinary hereditary phenomena, which resolve themselves into a multitude of minute genes or factors of the most diverse kind, while sexual phenomena are universally manifested in but two general conditions as male or female.

Now every specific sex character must be inherited in the same way as any other character not influenced by the sexual states. The genes of such characters are subject to aggregation and segregation in fertilization and reduction, to displacements and crossing over, the same as any other factors which are not especially influenced by sexual states. The problem of sexuality does not lie there. Many experiments have shown that the sexuality, whether expressed as a male, female or neutral condition, of a cell, tissue, organ or individual belongs in the domain of physiological states of the protoplasm and that it is apparently conditioned and determined by physiological gradients. If this is true then sex determination and sex reversal may be brought about not only in the manner usual for the given species or given hereditary complex acting in a given environment but also in various other ways and at other times of the life cycle, whenever a proper change in the functional state is produced through environmental or ecological factors.

In sexuality, we have a condition in the structural protoplasm or in its inclusions corresponding in some way to positive and negative chemical or electrical states so very common in the inorganic world, but the real nature of these conditions or states is yet to be investigated. But in the meantime, the phenomena of sexuality can be properly classified and the ecological basis of determination and control discovered to a considerable extent before we have solved the more hidden processes and conditions on which a specific female, male or neutral expression depend. Genetics is then not the primary basis for an investigation of sexuality but is a merely incidental phase which must be taken into consideration in studies

on the higher animals and a very few plants. It is useless
to speak of homozygous and heterozygous conditions as
determining sexuality or its reversal when in the vast
majority of plants neither sex determination nor reversal
occur at the time when the chromosomes with their Men-
delizing factors are associated in fertilization or segre-
gated in reduction.

The evidence from plant taxonomy for the view that
sex determination and sex reversal are not at all de-
pendent on or even to any extent associated with differ-
ential hereditary constitutions is overwhelming, but lack
of time forbids the presentation of such evidence here.

The terminology of sex is not very definite and much
confusion no doubt exists because of a different use of
terms. We distinguish sexual states as distinct from the
characters expressed by the hereditary factors when such
physiological states are present. These states are of
various degrees of intensity and persistency. Sexual
states are primary or secondary. Primary states ap-
parently arise only in gametes which are entire cells or
nuclei or in the synaptic chromosomes during reduction
and the main characteristics of such a primary state are
the properties of attraction and fusion. For convenience,
dimorphic characters of the gametes are called primary
sexual characters without reference as to the actual cause
of their origin which may perhaps be due to secondary
sexual states the same as in the case of secondary sexual
characters. Primary sexual states may arise in typical,
isogamous or heterogamous gametes or in only slightly
modified cells as in the conjugatae and typical fungi.
The attractive or sexual states which arise in the synaptic
pairs of chromosomes may occur at three different points
of the life cycle; first, at the germination of the zygote,
usually followed by a haploid sexual generation; second,
just before the formation of gametes, usually followed
by a diploid sexual generation; or third, in an alternation
cycle, at the end of the normally diploid sporophyte when
non-sexual spores are produced which give rise to a hap-

loid, gametophytic generation. Secondary sexual states
arise in cells outside of those destined to become gametes
but do not attain to the property of attraction and fusion.
They may, however, have a profound dimorphic influence
on the expression of certain hereditary characters of the
cell, thus giving rise to secondary sexual characters.
Some cells in the primary sexual state may pass by
parthenogenesis into a secondary or neutral state again.

In studying sexual phenomena, a clear understanding
of the sequence of events in the life cycle is of funda-
mental importance in order to understand the succession
of functional states, the changes of sexual conditions and
their relation to the shiftings of chromosomes and other
cell structures. In making such a study of the plants with
a typical antithetic alternation of generations, it becomes
evident that there is normally but one stage of sex deter-
mination in the life cycle, which stage may, however, con-
sist of a series of male and female determinations or their
reversal. If the sex is determined in the gametophyte,
which is usually haploid, there is no maleness or female-
ness in the sporophyte. If the sex is determined in the
sporophyte, which is usually diploid, it remains un-
changed through the reduction phase, both sides of the
reduction being of the same sex and the following gameto-
phytes are normally always unisexual. Roughly speak-
ing, one may say that there are about twelve main points
in the life cycle where sex determination may take place.
Of these twelve successive points of sex determination,
there are two in which the time of determination coin-
cides with chromosome shiftings. In plants with unisex-
ual gametophytes and homosporous sporophytes, the time
of sex determination coincides with reduction; in plants
with unisexual gametophytes and diecious sporophytes,
the time of sex determination apparently coincides with
fertilization, although it is possible that in this case the
determination takes place in the egg before fertilization
or some time after fertilization is completed. That the
determination of sex in the case of unisexual haploid

gametophytes is a mere coincidence in most cases can be shown by such examples as the ostrich fern and the common horsetail where the sex as determined may be easily reversed in the mature individual, the female to the male and the male to a certain extent to the female.

To determine the nature of the sexual condition in the higher plants, I used various diecious species, and since the sporophytes of the higher plants are extremely indeterminate in development, they make unusually favorable objects for experiment. The same is true of the indeterminate gametophytes of the Bryophytes and some Pteridophytes. But the higher animals, aside from the possible influence of the apparently sex-producing allosomes, are very unfortunate objects for such experiments because of the decided determinate development of the individual and most of its organs. Yet in spite of these conditions practically complete, functional sex reversals are being reported especially in the case of birds. In my experimental work on sex control and reversal, some species have proved very plastic and amenable to control, while others showed only a slight response to the ecological factors employed.

In *Arisaema triphyllum* (L.) Torr., which is a diecious plant with a variable percentage of intermediates, sex was almost absolutely controlled in the individual through a control of the nutritive environment. Abundance of moisture and manure with normal leaf surface throws the staminate and intermediate plants into the female condition and continues the carpellate individuals in the same condition, while a rather dry environment with a poor or ordinary soil and a reduced leaf surface throws the carpellate and intermediate plants into the male condition and continues the staminate plants in the same condition. These reversals can be produced back and forth repeatedly. *Arisaema dracontium* (L.) Schott. has a hereditary constitution that under the normal environment gives rise to staminate individuals and monecious individuals. I have never found pure carpellate in-

dividuals, although a few cases approached such a condition. Under the usual environment of the species, there is always a great excess of staminate plants. The species is preeminently a masculine species. Experiments of the same nature as those carried on with *Arisaema triphyllum* showed that the staminate individuals change to monecious individuals and monecious individuals change to staminate individuals.

Extensive experiments on the common hemp (*Cannabis sativa* L.) have shown that sex reversal results by changing the length of the daily illumination period. Hemp planted at the normal time in early spring develops as pure carpellate and pure staminate individuals with about equal numbers of the two sexes. With the variety used in the experiments no intermediate individuals whatever appeared under the normal environment. But when planted in rich soil with changing light period from August 15 to December 25 and from this date to May 1, the plants showed increasing sex reversal in both directions, depending on the relative length of daylight. The amount of reversal is approximately inversely proportional to the length of daylight. This percentage can be predicted just as definitely as any Mendelian ratio, when the proper environmental conditions are present. It is to be especially noted that in plants like hemp the same ecological factor or factor complex, contrary to what occurs with the nutritive control of Arisaema, produces reciprocal results, the female condition is reversed to the male condition in the carpellate plants and the male condition is reversed to the female condition in the staminate plants. There is also apparently a gradient of fixity or degree of intensity of the sexual state in the various individuals of a progeny which causes them to succumb to the environmental influence in the order of their several intensities, the weakest presumably responding first to the external stimulus. Thus in a patch of carpellate plants, some reverse promptly and some only after several months.

The percentage of intermediates or individuals that showed sex reversal to a greater or less extent among the carpellate plants for the successive planting dates was as follows:

Date	Percentage showing reversal	Total number of carpellate plants
August 15	0 per cent.	......
September 20	38 " "	72
October 19	53 " "	63
November 1	76 " "	120
December 1	80 " "	73
December 15	86 " "	29
February 1	65 " "	70
March 15	34 " "	59
May 2	0 " "	30
May 20	0 " "	24

The highest percentage of reversal for carpellate plants was obtained in two plats planted on December 19, 1919, in a north side room of the greenhouse where, in addition to the short period day, the light was less intense and the temperature somewhat cooler. One plat had 18 carpellate plants, 16 of which showed reversal and the other had 36 carpellate plants, 32 of which showed reversal. The sex reversal for these carpellate plants was, therefore, 88 per cent.

For the staminate plants the percentage of reversal was as follows:

Date	Percentage showing reversal	Total number of staminate plants
August 15	0 per cent.	......
August 19	15 " "	120
September 1	35 " "	20
September 20	57 " "	21
October 19	67 " "	40
November 1	90 " "	116
November 21	73 " "	73
December 1	93 " "	66
December 15	93 " "	46
December 20	90 " "	30
January 20	68 " "	25
February 1	65 " "	72

Date	Percentage showing reversal	Total number of staminate plants
February 20	56 Per cent	37
March 15	60 " "	50
March 20	33 " "	30
April 20	0 " "	28
May 1	2 " "	93
May 2	5 " "	18
May 20	0 " "	32

These records are from plantings covering several years, and the available daylight for any given period may be considerably less in one year than in another, which may account for the slight discrepancies in the table of staminate plants. The 93 per cent. reversal is the highest percentage obtained for staminate plants.

In the process of sex reversal from female to male or *vice versa,* the physiological condition must necessarily pass through a neutral point. This results in the abundant production of bisporangiate flowers, monstrosities and intimate sex mosaics in such hemp plants. These abnormalities appear also in the Arisaema intermediates and various other species used in the experiments.

Relative length of illumination period has similar effects on Japanese hop (*Humulus japonicus* Sieb. and Zucc.). As in the hemp, the same environmental complex causes reciprocal reversal and also the production of bisporangiate flowers, abnormal organs and intimate sex mosaics.

Thalictrum dasycarpum is a diecious plant which in its normal habitat shows sex intergrades quite frequently. These intermediates are of every conceivable degree, ranging up to individuals producing about an equal number of staminate and carpellate flowers or sporophylls. The question presented itself as to whether this series of sex forms represents so many types of hereditary constitutions or chromosome or factor arrangements which are responsible for the result. A simple experiment answered the question. Pure carpellate and pure staminate individuals were transplanted from their rich flood

plain habitat to rather dry clay soil. In two years both
the pure carpellate plant and the pure staminate plant
had developed into sex-intergrades. The carpellate plant
developed decided maleness and had many more stami-
nate flowers than carpellate ones. The notion of the fix-
ity of the sexual state, dependent on a specific heredity
complex, was thus shown to be entirely erroneous in this
case also. The cause of these different grades of sexual
expression is not found in specific hereditary constitu-
tions. The various types of intergrades as well as the
pure individuals apparently have the same hereditary
constitution which is of such a nature as to permit sexual
states to be easily changed.

Numerous other examples might be given from my own
observations and experiments as well as from those of
other investigators, but time permits of but one further
point in the discussion.

If the physiological view of sexuality is the correct
one, it becomes evident that cells or cell lineages must
be able to pass from one sexual state to another without
change of hereditary constitutions and such is actually
the case. In the dandelion (Leontodon), various varie-
ties or species have evolved to a condition where normal
synapsis does not take place except in rare instances.
Sears and others have shown that the plant usually passes
through its life cycle from generation to generation by
means of parthenogenesis with the diploid number of
chromosomes. Beginning in such a case with the vege-
tative cells of a sporophyte, such cells are in a neutral,
vegetative state in respect to sex. When flowers appear,
secondary sexual states arise and secondary female
characters are developed in the gynecium on the one hand
and secondary male states and secondary male characters
in the andrecium on the other. In the ovules the mega-
sporocytes divide without reduction, the female gameto-
phyte develops with the usual secondary female charac-
ters but with the double number of chromosomes. An
egg which is then also diploid is organized, but whether

a primary sexual state appears giving it the power of fusibility can not, of course, be determined in any specific case of parthenogenesis. It is well known that in mosses the double number of chromosomes is no barrier to the development of attractive properties and subsequent fertilization. The diploid egg develops the new, neutral sporophyte which again produces flowers. Passing through the andrecium cell lineage, the stamens show secondary male states and characters. In the microsporangia the microsporocytes may divide without reduction, with abnormal or incomplete segregation of chromosomes, or occasionally with a normal reduction, thus giving rise to normal microspores and male gametophytes with normal haploid spermatozoids. In this cell lineage, therefore, without any change in chromosomes or hereditary constitution, the sexual state passes from a neutral condition to a female condition, to a neutral condition, to a male condition.

Blakeslee and Belling have recently reported the interesting case of haploid sporophytes in the jimson-weed (*Datura stramonium* L.). They consider the evidence good that such haploid sporophytes arise through parthenogenesis. The haploid cell lineage will, therefore, show the same changes in sexual states as that of the diploid Leontodon lineage. Proceeding through the same tissues we have: neutral, diploid sporophyte, secondary female state and female characters in the gynecium, sporocyte, reduction (without change of sex), female gametophyte and primary female characters in the egg (and perhaps also primary female state for a period). Through parthenogenesis the neutral, vegetative, haploid sporophyte is produced which leads on to secondary sexual states in the flower, female characters in the gynecium, male characters in the andrecium. In rare instances normal, haploid, non-reduction divisions take place in the sporocytes leading on to normal male and female gametophytes, respectively, with normal sperms and normal eggs, which in consequence of the primary sexual states arising in

them may conjugate and produce a diploid generation
again. Thus it is seen, as is perfectly plain from the
taxonomic evidence, that the presence of a diploid or
haploid number of chromosomes has nothing to do with
the origin of primary and secondary sexual states and
characters.

In my photoperiodic experiments with hemp such a
case of definite sexual changes appeared in a rejuvenated
carpellate plant. A certain short-light individual was
pure female in expression and produced numerous nor-
mal carpellate flowers and seeds. After it became quite
decrepit, it was rejuvenated from side buds by means of
continuous light and passed into a new vegetative phase
of growth. In passing through this reversed physiologi-
cal gradient, after growing for about a foot, it began to
bloom again and produced a considerable number of
flowers and, as some would say, *mirabile dictu,* these flow-
ers were all absolutely of the pure staminate type. The
pure female had for the time being changed to a pure
male, when judged by its reproductive behavior. It soon
stopped blooming, however, because of the continuous
light effect and the terminal end of the inflorescence con-
tinued a normal vegetative growth. After several months
the light was turned off and it promptly passed into the
blooming condition again. At this third blooming period,
every flower was pure carpellate! After some seed had
set it was cut back to some extent, rejuvenated again, and
after a period of vegetative growth was beginning its
fourth period of anthesis, but died, because of previous,
improper pruning, before the flowers were fully ex-
panded. Some of the flowers had well-developed stigmas,
but none showed recognizable staminate structures, as
might have been anticipated from the date of this bloom-
ing, December 15. If the plant had lived, some maleness
might still have developed. Dandelion and jimson-weed
are bisporangiate plants; here we have a strongly di-
morphic, diecious species—sexually dimorphic in its vege-
tative organs and in its reproductive organs, and strongly

dimorphic in its physiological processes—a carpellate plant of which developed successively a vegetative tissue that at first produced secondary and then primary female states and characters, next secondary and primary male states and characters, and then secondary and primary female states and characters again. The succession of functional states leading to the several zones of sexual expression is exactly similar to what takes place in numerous monecious species in which the flowers appear in a definite zonal succession, but with this difference in the vegetative parts. In normal monecious plants the vegetative phase is neutral; in this carpellate plant the vegetative phase was in the first period of growth typically female.

In organisms which have allosomes, the most that can reasonably be claimed for the allosomes is that they may be sex-producing. The given heredity complex may produce a higher or lower metabolic level and thus control sex. They can plainly not be sex-determining. But if they are merely sex-producing rather than sex-determining, then the sex of an organism or any of its parts containing such allosomes is subject to reversal through a control of its physiological states by external factors the same as in organisms without allosomes. Whether such reversal is more difficult to produce in allosome-containing cells is a question in view of the well-known fact that with a change of hormones in the circulatory system of some of the higher animals the sexual expression changes readily, and apparently to as great an extent as the determinate nature of the animal will permit. In view of such facts as normal hens changing completely or functioning as roosters, etc., it is evident that allosome-containing organisms are suitable subjects for physiological experimentation with a view to producing sex reversals in either direction the same as organisms without such allosomes, although it may perhaps be more difficult to produce the change. But the very fact that allosome-containing organisms change their sexual states and sexual expres-

sions as readily as they do throws considerable doubt on the hypothesis that they are even primarily sex-producing. It may be a mere coincidence. They may be merely sex-indicating and after a case of sex reversal they can not even be that. The sex of the egg may be determined even before fertilization takes place. Many botanists formerly thought that a diploid number of chromosomes was the cause of sporophytic expression and a haploid number the cause of gametophytic expression, but we now know from a study of apogamy and apospory and from experiments on mosses, as well as from such haploid and diploid parthenogenetic developments as Datura and Leontodon, that the doubling and reducing of chromosomes in the higher plants not only has no direct relation to sex determination, but also that these processes have no causal relation in the production of the profoundly dimorphic gametophyte and sporophyte.

The conclusion is then that sex in organisms is primarily dependent on physiological states, that these states are subject to change and reversal through ecological factors and that, therefore, sexuality is a subject for experimental investigation and one of the problems that physiological ecology alone can properly solve.

SEX IN RELATION TO CHROMOSOMES AND GENES

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DURING the three years since the report at the Toronto meeting (Bridges '22) considerable new information has accumulated with regard to the series of different sex-types that has arisen in the breeding work with Drosophila (Table I). Each of these different sex-types is the result of a particular combination of chromosomes. They occur principally among the offspring of females that are triploids, that is, that have three X-chromosomes and

three of each kind of autosome. The possession of an extra X and at the same time of an extra set of autosomes does not change this individual in its sexual characters from the normal type of female. However, in gametogenesis the 3N group is an unstable one. Each egg receives a full set of chromosomes and a full set goes to the polar body. The members of the extra set are distributed between the egg and the polar body in all possible combinations. Thus, a common type of egg of a 3N female has an extra set of autosomes. If we represent a set of autosomes by A, this egg can be formulated as $X + A + A$ or $X,2A$. When such an egg is fertilized by an ordinary sperm, that can be formulated $X + A$, the zygote is $2X,3A$. This type of zygote develops into an intersex, that is, into an individual that is neither male nor female but an intermediate, or rather a mixture of male and female parts, very similar in type to the intersexes that Goldschmidt has worked with so extensively in *Lymantria dispar* (Goldschmidt '20).

TABLE I

RELATION OF SEX TO CHROMOSOMES IN DROSOPHILA MELANOGASTER

Sex Type	X (100)	A (80)	Sex Index	Interval	$X = -6$ $A = +2$
Superfemale	3	2	1.88	50%	-14
Female 4N	4	4	1.25	.....	-20
Female 3N	3	3	1.25	.....	-12
Female 2N	2	2	1.25	.....	-8
Female N[1]	1	1	1.25	50%	-4
Intersex ♀ type	2	3	.83	.....	-6
Intersex ♂ type	2	3–IV	.83–	33%	-6
Male	1	2	.63	50%	-2
Supermale	1	3	.42	.....	0

The interpretation of these intersexes in terms of genes carried in the chromosomes was made possible as the result of the very extensive studies of the manner in which ordinary characters are determined, and especially from the study of the contrasted character changes

[1] The haploid type has not been discovered.

brought about on the one hand by the loss, and on the other hand by the gain, of one of the small round chromosomes, the fourth chromosome. From such studies the view had been reached that each character of an individual is the index of the point of balance in effectiveness of a large but unknown number of genes, some of which have a tendency to change development in one direction and others in the opposite (Bridges '22). This conception of "genic balance" was applied to the sex characters of the intersexes as follows: In chromosome constitution the intersexes differ from females only in that they have an extra set of autosomes. This proves that the autosomes are concerned with the determination of sex. Moreover, they are male-determining in their action, since the addition of a set of autosomes causes the female to assume male characteristics. That is to say, in the autosomes there are genes that tend to produce the characters that we call male, and these are more effective, either through greater numbers or through greater potency, than the total of autosomal genes tending to produce the alternative characters that we call female. On the other hand, the X has a net female tendency, as shown by the fact that the addition of an X to a male group changes the individual into a female. The net male tendency of a set of autosomes is less than the net female tendency of an X. This is seen in the fact that in the individual in which there are two of each, namely 2X,2A, the female genes outweigh the male and the result is a female. If we represent the net effectiveness of the female tendency genes in the X by 100, then we should represent the net male effectiveness of a set of autosomes by some lower number; let us say 80. In a 2X,2A individual the ratio of female effectiveness to male effectiveness is 200:160, or 1.25 to 1; and on this formulation the sex index of 1.25 corresponds to the normal female. In the X,2A individual the ratio of female to male effectiveness is 100:160; or the sex index of a normal male is 0.63. In the 2X,3A intersex the ratio is 200:240, and the sex

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index is 0.83, which is intermediate between the indices for female and male. In the 3N female the ratio is 300 : 240, and the sex index is 1.25, exactly the same as in the normal female. This identity of sex indices for the 3N and 2N forms corresponds to the observation that there seems to be no strictly sexual differences between them. The larger size, coarser texture of eye, etc., of the 3N can be directly attributed to the changed volume of the nucleus, and are not sexual in nature.

Another type of egg of the 3N ♀ is $X + X + A$; and this, fertilized by a normal XA sperm, gives a 3X,2A individual with a sex index of 1.88, which is 50 per cent. higher than that of the normal female. This constitution corresponds in fact to the "superfemales" that occur in these cultures and elsewhere. The superfemales are much delayed in development, are rarely able to live and are probably completely sterile.

Conversely, an $X + A + A$ egg, fertilized by the type of sperm that does not carry an X, gives an X,3A zygote with a sex-index of only 0.42. This type of individual was expected to be more male-like than an ordinary male; and such individuals were looked for among the offspring of 3N females. At first none were found; but presently it was discovered that very late in the cultures an occasional example of a distinct type of male occurred. These so-called "supermales" are likewise sterile. Recently, cytological proof has been secured that this type of sex has the constitution X,3A, which agrees with the genetical evidence previously secured.

It was observed that the intersexes showed considerable variation and seemed to form a bimodal class. And since the cytological investigation had showed that some intersexes had three and others only two of the small round fourth-chromosome, it was guessed that the more male-like mode corresponded to the full trio of fourth chromosomes, while the more female-like mode corresponded to the cytological type that lacked one fourth chromosome. An effort has been made to secure cyto-

logical evidence on this point. But this evidence is in-
conclusive; as is also that from an attempt to make a
genetic test of the number of fourth chromosomes present
through use of the fourth-chromosome mutant character
eyeless. At present extra fourth chromosomes are being
artificially inserted into the intersexes by continually
crossing 3N mothers to males known to have an extra
fourth chromosome. Contrariwise, in other lines of inter-
sexes, fourth chromosomes are being diminished in num-
ber by continually mating 3N mothers to males known to
lack one of the two fourth chromosomes. Similarly, the
superfemaleness of the 3X,2A individuals might be re-
duced or be increased by matings with triplo-IV males or
with haplo-IV males. For this experiment females are
being used whose two X-chromosomes are permanently
attached to each other (L. V. Morgan '22), and hence that
give through non-disjunction a very high proportion of
3X-superfemales. Present indications, from the uncom-
pleted experiments, are slightly contradictory, but tend
to a conclusion which is the opposite of that earlier re-
ported as probable on the basis of the slight evidence then
available (Bridges '22). When the number of fourth
chromosomes is three the intersexes are more female-like,
and when the number is two they are more male-like. The
fourth chromosome has a net female tendency, similar to
that of the X and different from that of the other auto-
somes. By variation in the number of fourth chromo-
somes it is possible to have a fringe of minor sex-types
about each of the major types of sex difference.

The list of sex-types has been enlarged by the discovery
of tetraploids, or 4N individuals. These are females,
quite identical with normal females in sex characteristics.
The tetraploid arose in a stock of triploids; and was de-
tected only by the strikingly different offspring given. A
female supposed to be 3N was selected from the 3N stock
and outcrossed to a normal male. All the offspring were
triploid females (about 30) or triploid intersexes (about
20). There were no 2N offspring or supersexes. It was

seen that this result might be produced if the mother were 4N instead of 3N. For in that case all the reduced eggs would be 2N; and these fertilized by X-sperm would give 3N females, and fertilized by Y-sperm would give 2X,3A intersexes.

Before the discovery of this 4N individual, an expectation that it would occur had arisen from several facts. Thus, in the three years following the discovery of triploidy there had been found no less than twenty-five instances of the new occurrence of triploidy. This very high frequency was paralleled by cytological observations that give the explanation of the origin of triploids. In three separate preparations of ordinary 2N females it was found that a portion of an ovary was constituted of markedly larger cells; and in two of the individuals some of the giant cells were in division, and the chromosomes could be counted as 4N. Evidently there had been in some oogonial cell a division of the chromosomes that had not been followed by division of the nucleus and cytoplasm. The resulting tissue was tetraploid, and any reduced gamete would be 2N. Such a 2N gamete, fertilized by a normal sperm, would account for each of the twenty-five recurrences of triploidy.

Furthermore, in examining sections of intersexes, two individuals were found in which similar cysts of even larger cells were present. In one of these cysts divisions were occurring; and the chromosomes were clearly 6N. A 6N cyst in a 3N female would give, upon reduction, 3N eggs, which, fertilized by X sperm, would give the expected 4N type of female.

Soon after this first case of 4N female a second similar case was found. Also L. V. Morgan found a third case, and was able to prove by genetic tests that four separate X-chromosomes had been present (in press).

The fact that 4N individuals are females, not modified as to sex, has important bearings on our ideas as to the way in which genes interact to produce their effect. The view adopted here is that in general the effectiveness is

in proportion to number of genes, and the significant point is the *ratio* between sets of genes that tend to produce alternative effects. On this view we find a ready explanation for the fact that such diverse forms as 2N, 3N and 4N individuals are precisely alike in their sexual characteristics; for in all these forms the effectiveness of both contending sets of influences has been doubled, trebled or quadrupled; and the ratio remains constant.

But a system of formulation different from the ratio type has been adopted by Goldschmidt in dealing with the intersexes produced in the course of his brilliant work on racial crosses of *Lymantia dispar*. To the male tendency of a particular race he assigns a positive value that is proportional to the strength of the male-determining gene of genes. To the female tendency he assigns another value also proportionate to the strength of the female-determining gene or genes. He then assumes that when in an individual the male value is greater than the female value by a certain number of units the individual is 'a male, and that, conversely, when the female value is greater than the male by this same number of units the individual is a female. The locus of the male tendency gene (M) is in the "Z-chromosome" of which two are present in the male and one in the female. The female tendency is strictly maternally inherited; and hence the locus of the F genes is in the W-chromosome that descends from mother to daughter. The F gene is supposed to exert its influence on the cytoplasm of the developing egg; and hence, although the male has no W-chromosome, he is supposed to have a definite female tendency that was impressed upon the cytoplasm of the egg and that persists throughout development. For a "weak" race the value assigned to F is 80, and to M, 60. In the WZ individual the cytoplasmic $\boxed{F}$ of 80 exceeds the M value of 60 by "the epistatic minimum" of 20 units, and the individual is a female. Likewise, in the ZZ individual the $\boxed{F}$ is 80, but the net M value is twice 60 or 120, with an excess in the male direction of 40 units. For a strong

race both F and M are higher, for example, 100 and 80, but the arithmetical relation between the values of F and M would still govern the sex of the individual. In a cross between a weak female and a strong male the ZW individual received an $\boxed{F}$ of 80 from the mother and an M of 80 from the father. The values are thus balanced midway between the excesses necessary for a female on the one hand or a male on the other, and the result is an intersex. This far the formulation is satisfactory; but when extensive series of crosses are compared, and an attempt is made to give values to the F and the M of each race that will hold throughout the entire range of experiments, this attempt is rather unsuccessful. It seems to me that a reformulation on the ratio instead of the algebraic basis would give a series of consistent indices without running counter to the very valuable physiological ideas that Goldschmidt has developed.

In the table of sex-types of Drosophila the haploid individual is entered with the index 1.25, the same as that of the 2N, 3N and 4N females. Unfortunately the haploid individual has *not* been discovered. But it is plain from the view just given that the expectation for a haploid *Drosophila melanogaster* is that it would be female in sex character. Accordingly, it is necessary to assume that the sex-determining mechanism here is essentially different from that in the bee and similar forms in which the haploid individual is a male. To me, sex-determination in the bee is the outstanding unsolved puzzle, although before the development of the idea of genic balance it seemed one of the clearest and simplest of cases. If it is true that the male is a haploid individual, then one would suppose that the diploid individual should likewise be a male, since the ratios among the sex-determining genes are not different in the two cases.

Schrader and Sturtevant have attempted a reconciliation of the cases of Drosophila and the bee by use of Goldschmidt's algebraic formulation. They assign a positive value, *e.g.,* $+2$, to each A and a negative value,

e.g., — 6, to each X.   It is then assumed that the effective
relation is the algebraic sum of the values of X and A,
as given in the column to the right in Table I.   On this
view the haploid might be a male.   But this system has a
difficulty in that the intervals between successive indices
do not correspond very well with the observed differences·
between the sex grades.   Thus the smallest observed in-
terval in fact, that between the 3N and 2N individuals, is
represented by a difference of 4 units, while the very
great interval between the male and the female is repre-
sented by only six units.   At that time the 4N type was
not known; and when it is added to the series, the fit is
very poor on the algebraic system and very good on the
ratio system.   I repeat that I do not regard the case of
the bee as interpretable on the same basis as Drosophila
so long as the present account of the mechanism for the
bee is unchallenged.   At present the difference between
haploid and diploid sexes must be referred to the same
type of determination as that responsible for the larger
size, rougher texture of eyes and other slight changes
that distinguish the 3N from the 2N individual.

But outside of the cases like that of the bee, it seems
probable that the ratio type of interaction is the general
mode.   In evidence of this may be cited the vast array
of monoecious plants in which the sex relations in the
triploid and tetraploid remain the same as in the diploid.
Of course there is high sterility in the triploid forms on
account of the instability of the 3N group in meiosis, and
the consequent production of inviable gametes or zygotes.
Among the most striking confirmations of this ratio view
of genic balance is seen in the mosses, through the bril-
liant work of the Marchals, Schweitzer and von Wett-
stein.   For example, they find in a moss with separate
sexes that a 2N gametophyte that combines 2 female
groups of chromosomes is a pure female, like the haploid
female plant (Table II).   Likewise the 2N gametophyte
that combines two male groups of chromosomes is a pure
male plant, like the haploid male plant.   But a 2N gameto-

TABLE II

SEX TYPES IN A DIOECIOUS MOSS

Sex Type	X (100)	X' (50)	A (80)	Ratio (X + X') : A	Sex Index
Female { 2N	2	...	2	200 : 160	1.25
⎩ N	1	...	1	100 : 80	1.25
Proterogynous Herm. 3N	2	1	3	250 : 240	1.04
Protandrous Herm. { 4N	2	2	4	300 : 320	.94
⎩ 2N	1	1	2	150 : 160	.94
²Protandrous Herm. 3N	1	2	3	200 : 240	.83
Male { 2N	...	2	2	100 : 160	.63
⎩ N	...	1	1	50 : 80	.63

phyte that combines a male and a female group is no longer a single-sexed plant but is a protandrous hermaphrodite. Furthermore, the 4N gametophyte that combines 2 male chromosome groups with 2 female groups (FFMM) is a hermaphrodite like the FM hermaphrodite. But a triploid form in which two groups are female and one is male is a hermaphrodite that is strongly protogynic instead of protandrous. On the other hand, in working with monoecious mosses where the haploid group is a hermaphrodite, then all haploid, diploid, triploid and tetraploid plants were hermaphrodite without distinction, as they should be from their possession of the same ratio of female to male determiners.

A series of sex-indices, similar to those for Drosophila, can be fitted to the dioecious mosses, as shown in Table II. Here it is assumed that there is a pair of chromosomes, X and X', whose difference accounts for the difference between the female and male types. It is assumed that in both these sexes the net effect of the other chromosomes, that may be represented by A, is male-determining. Then, since the X,A type is a female, the value for A must be less than that of X, *e.g.*, X = 100 and A = 80. Likewise, since X',A is a male, the value X' must be less than that of A, *e.g.*, X' = 50. Also, since the FM plant is a hermaphrodite that resembles the normal male more

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² This type is not reported as realized.

than the normal female, $X + X' < 2A$. And, since the FFM plant is a hermaphrodite that resembles the normal female more closely, $X + X + X' > 3A$. We have thus five limiting equations for the three values X, X' and A. As the table shows, the assigned values of 100, 50 and 80 are possible, although there may be other slightly different values that would give a set of indices whose intervals would correspond even more closely to the observed differences than do those given.

The same conformity to the ratio rule seems to be true in the haploid, diploid, triploid and tetraploid daturas, as far as I can gather. But on the genic balance view each of the twelve kinds of chromosomes of Datura might have a distinctive internal unbalance of the sex-controlling genes, similar to the unbalance in the fourth chromosome of Drosophila. In Datura there is a full series of forms that differ from the 2N by the addition of a particular extra chromosome. If any of these twelve kinds of chromosomes contain more effective male tendency genes than female tendency genes, or vice versa, then one may well expect to discover that some of Blakeslee's "Apostles" and "Acolytes" have atypical sex-relations.

LITERATURE

Blakeslee, A. F.
>1922. Variations in Datura, due to changes in chromosome number. Am. Nat. 56: 16–31.

Bridges, C. B.
>1922. The origin of variations in sexual and sex-limited characters. Am. Nat. 56: 51–63.

Goldschmidt, R.
>1920. Untersuchungen über Intersexualität. *Zeit. f. ind. Abst. u. Verer.* 23: 1–197.

Morgan, L. V.
>1922. Non-criss-cross inheritance in Drosophila melanogaster. *Biol. Bull.* 42: 267–274.

Schweitzer, J.
>1923. Polyploidie und Geschlechterverteilung bei Splachnum sphericum Schwartz. *Flora* 116: 1–72.

Wettstein, F. v.
>1924. Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage. *Zeit. f. ind. Abst. u. Verer.* 33: 1–236.

SEX AND THE PARTHENOGENETIC-BISEXUAL CYCLE

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AMONG the groups of animals which reproduce both parthenogenetically and by fertilized eggs, there are two general types. In animals of one type there is but one kind of egg, capable of being fertilized, but capable also of developing without fertilization. Animals of the second type produce two kinds of egg, one kind capable only of parthenogenetic development, the other either requiring fertilization or being capable of being fertilized. It is only in the latter of these two types that the reproductive changes may be described as cyclical. Cycles involving two types of eggs, of the sorts just described, are found chiefly in the rotifers, cladocera and aphids. It is to the problems of sex in these groups that your attention is invited.

The cycle of reproduction in the rotifers is more definite and better understood than in either of the other groups. From the fertilized egg of *Hydatina senta,* a rotifer whose cycle is well known, hatches a female whose offspring, parthenogenetically produced, are likewise females. These females of the second generation may all be, like their mother, capable of producing only obligatorily parthenogenetic eggs which develop into females; or some of them may produce smaller eggs which are facultatively parthenogenetic, yielding males if unfertilized, but females if fertilized. There are thus two kinds of female, wholly distinct from one another though alike in gross appearance, which have been variously named male-producers and female-producers, or sexual and parthenogenetic females, or recently, by Storch (1924), mictic and amictic females. All these names are objectionable in that the terms applied to the male-producing type refer to but one of the two possible

destinies of its eggs.   These two types of female may oc-
cur together in the second generation, that is, among the
daughters of the female hatching from a fertilized egg,
and in any generation thereafter as long as partheno-
genetic reproduction is maintained.   The facultatively
parthenogenetic or mictic females are usually in the
minority, but their relative numbers vary within wide
limits.   Most of the investigations of the relation of en-
vironment to mode of reproduction in rotifers have been
concerned with the varying proportions of the mictic
females.

The cycle of the aphids is similar to that of the rotifers,
except that the mode of reproduction is less intimately
related, as is pointed out later, to the sex of the indi-
viduals.   In *Macrosiphum solanifolii,* a typical species,
the exclusive mode of reproduction during the summer
is parthenogenesis, the parthenogenetic females being
either winged or wingless.   In warm regions partheno-
genesis may continue during the entire year, but in colder
climates sexual females and males appear in late summer
or autumn.   The sexual females are recognizably differ-
ent from the parthenogenetic ones, and their eggs must
be fertilized in order to be laid or to develop.   Similarly,
in most other species, a female is exclusively partheno-
genetic or wholly sexual, but in rare cases both kinds of
eggs have been produced by the same female.

The cycle of the cladocera is not unlike those of rotifers
and aphids, but the types of female are less sharply
defined.   Indeed, in *Simocephalus vetulus,* which has
been extensively used in experimental work, there is only
one type of female.   In this species, according to Green
(1919), any female after the stem mother, which hatches
from a fertilized egg, may produce either ephippia en-
closing the sexual eggs, or, by parthenogenesis, males or
females, the latter of which again have all three of these
possibilities.   One female often produces two of these
kinds of offspring, and sometimes produces all three.
If a female produces only one kind of offspring, this kind
is almost invariably the parthenogenetic female.

It is perhaps owing only to the liberal conception and wording of the subject of this symposium that a discussion of sex in the groups exhibiting cycles like these is permitted a place. To many biologists the problems raised by the rotifers, cladocera and aphids, or at least the problems whose solution has been most assiduously sought in those groups, have seemed to be those only of sex production, not sex determination, notwithstanding that the term sex determination has been applied to all of them repeatedly. If, before the end of this discussion, I shall have tried to indicate that the ancient practice of referring to the factors which bring on the generations of males and sexual females, in the groups named, as factors of sex determination, was not entirely incorrect, it will not be because the phenomena then commonly referred to should, in my opinion, be regarded as sex-determining. In the rotifers, the entrance of the bisexual phase of the cycle was made known by the appearance of males, where all had been females before. Though it is now well known that the females are of two kinds and that both kinds must be produced before males appear, that was not patent at first, since all females looked alike. In the aphids and cladocera the sexual females in many species closely resemble the parthenogenetic females, and may indeed sometimes be the same individuals. In these groups also the first readily recognizable sign of the sexual forms was the occurrence of males. It is not surprising that, before the nature of the cycle was well understood, the causation of the sexual phase should have been regarded as sex determination. Whether it should be surprising that the name remained, after the phenomena were shown to be different, depends on one's opinion of human nature.

Some justification for the name sex determination remains, however, even when sex production is kept in the foreground. In the rotifer. *Hydatina,* for example, there is a definitely fixed relation between sex production and the determination of one of the sexes. Thus, the male

is invariably the offspring of the sexual female. The only way to determine the sex of a male in *Hydatina* is first to determine a sexual female and then wait a generation. Male determination is not, it is true, necessarily final, for the sexual female may be impregnated when young, whereupon the same eggs produce female offspring. Reversal of male determination does not, of course, deprive the first event of its determinative significance. Indeed, if production of a sexual female is not determination of the male in the following generation, in *Hydatina*, then there is no male determination in this rotifer.

There are indications that in the aphids also sex production carries with it certain elements of sex determination. It has been shown (Shull, 1918b) in some lines of the potato aphid, *Macrosiphum solanifolii*, that in the summer winged individuals appear during successive parthenogenetic generations with increasing frequency. The cause of this gradual change, when it occurs, has not been ascertained, but judging from experimental work on wing production in other aphids it is probably due largely to environmental factors. When, in the experiments referred to, the sexual forms appeared in the fall, the sexes showed an evident relation to wings in the preceding generation. Most of the males were born of wingless mothers, while the sexual females were produced chiefly by winged females. There was no rigid relation between these forms, since the same wingless females which produced males also frequently or occasionally produced winged or wingless parthenogenetic females or sexual females as well; and the winged females which gave birth to sexual females also yielded winged and wingless parthenogenetic females and, rarely, males. Notwithstanding these varied capacities, it was nevertheless true that the wingless parthenogenetic females, if they produced sexual forms at all, produced chiefly males, while the winged parthenogenetic females gave birth mostly to sexual females. A like dependence upon wings

or their absence has been observed repeatedly in other aphids and is perhaps a general condition. Indeed, systematic writers are accustomed to distinguish separate kinds of parthenogenetic females whose function is the production of the sexual forms, and to apply distinct names to them. Exceptions are recorded by some of these writers, however, and it appears likely that in aphids in general a cycle like that of *Macrosiphum* is common; that is, that there is no type of individual rigidly distinct, even in a physiological sense, as the mother of either of the sexual forms, but that sexual females are produced predominantly by winged mothers, males by wingless mothers. Nevertheless, the cycles of aphids are extremely varied (Patch, 1920).

Under the circumstances described for *Macrosiphum solanifolii,* any condition which brings on the sexual phase while the wingless females are in the majority favors the production of males, while if the sexual phase is delayed until winged females are predominant, females are favored. A probable result of these alternative possibilities is found in the nature of the sexual phase of the potato aphid in our experiments in two recent years. Several years ago, the sexual phase began in the first week of September, males appearing first and in considerable numbers. In the fall of 1924, however, sexual forms did not appear until the middle of October, and males were rare. Even if the gradual increase in the number of winged parthenogenetic females which was observed in our experiments, particularly in one season, should prove not to be a regular occurrence, as may easily be the case if the wing production in this species is environmentally influenced, the time of incidence of the sexual forms would still be highly significant in determining the relative frequency of sexual females and males. Appearance of the sexual forms during a period of few wings would favor males, while abundance of winged females at that time would increase the proportion of sexual females.

The physiological differences between the several types of individual in this species are under investigation, and it is too early to arrive at a conclusion, but it seems at present not unlikely that one or more of the fundamental differences between winged and wingless females are of the same order as those that distinguish the sexual from the parthenogenetic forms and males from sexual females.

Enough has been said regarding the cycle of the rotifers and plant lice to show that, although the expressions "sex production" and "sex determination" properly designate two different phenomena, they are more or less closely related to one another. It is not unlikely that some elements of the two may be identical. That a similar relation exists in the cladocera is not improbable. Varying degrees of intersexuality, affecting both the primary and secondary characters, described by Banta (1918, 1921) and La Vaulx (1919), and alternations between parthenogenetic and bisexual reproduction are both readily effected by environmental conditions; and according to La Vaulx (1922) intersexuality may be induced by at least one environmental factor which several investigators have observed to affect the mode of reproduction.

The form of the cycle, by which is meant the frequency, duration and magnitude of the periods of sex production, is subject to great fluctuation in all these groups. There are inherited differences between parthenogenetic lines, some of which produce few sexual forms, others many, under the same conditions. Within a given line also great modification can be effected by artificial means. In the rotifers, sexual reproduction may be postponed or even prevented by a variety of chemical substances in the medium; or it can be hastened and increased by the use of green protozoan food or, according to our results (Shull and Ladoff, 1916; Shull, 1918a) which Whitney (1917, 1919) was unable to confirm, by increasing the oxygen content of the water. In cladocera, temperature, food

and excretions in the medium have been held responsible,
either directly or indirectly, for similar advancement or
postponement of the sexual phase. In aphids, an old
conjecture that low temperature brings on the sexual
phases has been confirmed in a sense by several experi-
menters, in whose minds there lurks, however, a suspicion
that the effect of the low temperature is due to its action
on the food of the insects. Length of the period of day-
light has likewise been thought (Marcovitch, 1923; David-
son, 1924) to influence the cycle, though obviously this
may only indirectly influence the aphids through their
nutrition. The agents used in regulating the appearance
of the sexual forms have been so varied, especially in the
rotifers, that no characteristic common to them can be
discovered. Their effect must be conceived, therefore,
either as a general modification of metabolism, or as
being exerted upon some fundamental mechanism which
is readily disturbed by a variety of means.

That there is a relation between metabolism and the
production of the sexes in *Hydatina senta* has been shown
repeatedly in experiments. Periods of abundant sexual
reproduction tend to alternate with periods of almost
exclusively parthenogenetic forms (Shull, 1915), an alter-
nation which has been mentioned at least by implication
by several authors and probably witnessed by all investi-
gators of this species. Periods of abundant sexual forms
have been invariably characterized by rapid growth,
rapid reproduction, large families and relative indiffer-
ence to the amount of care bestowed by the experimenter
upon the rearing of them. Signs of this increased meta-
bolic activity precede the appearance of the sexual forms,
indicating that, if the two events stand in the relation of
cause and effect, the increased metabolism is the cause,
and that, if both are effects of the same cause, increase
in the rate of metabolism results more rapidly than does
sex production. This observation has been made, not
only by experimental workers, but by those whose inter-
est is systematic or in the field of freshwater biology.

Wesenberg-Lund (1923), for example, in a search for males in numerous species used this method of determining when to look for them. He states that as early as 1898 he had shown that "before a sexual period sets in the species in a given locality will very often increase enormously in number. This observation has been corroborated by almost all later authors."

A striking parallel to the periodical occurrence of sexual reproduction is found in *Paramecium*, as described by Hopkins (1921). Clones of this protozoon differ from one another in their readiness to conjugate. All of them exhibit a periodicity of conjugation, with fairly constant intervals between their periods, but different intervals in different clones. Conjugation is initiated by a period of unregulated division, and it is thought possible that whatever effect external conditions have on conjugation is exerted first on the rate of fission. Practically every point in this statement concerning *Paramecium* duplicates, with slight verbal changes, the corresponding statement for *Hydatina,* indicating that the metabolic relations of sexual reproduction are wider than the parthenogenetic-bisexual cycle.

What is the explanation of the phenomena so characteristic of the cycles in the rotifers, aphids and cladocera—the different types of females, the changes in the mode of reproduction, the increased physiological activity preceding the production of the sexes? Old-fashioned geneticists, among whom I count myself, when seeking explanations of sex and related phenomena, think early of chromosomes. They are entirely free to consider chromosomal changes, since the annoying and disconcerting discovery of one botanist that sex may be explained without chromosomes was probably conceived to relate only to plants. Unfortunately for any chromosome explanation of the cycles in question, the facts so far known regarding numbers speak against it. The first definite chromosome count in the rotifer *Hydatina senta* (Shull, 1921) set the diploid number at 12, alike in partheno-

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genetic and sexual females. Though reduction occurs in the sexual egg, this event is much too late to have any determinative significance. Tauson (1924) finds in *Asplanchna intermedia* that the diploid chromosome number is 24 (according to Whitney, 1924, it is 52), alike again in parthenogenetic and sexual female. *Asplanchna priodonta,* according to Storch (1924), has 16 chromosomes, the same in both types of female. In aphids, the parthenogenetic females which produce the sexes are like each other and like other parthenogenetic females with respect to chromosome number; and though males and females differ in the chromosome numbers through a type of non-disjunction, the decisive event occurs in an indeterminate proportion of eggs from one mother, dependent presumably, not upon chance, but upon some unknown factor perhaps environmental. In daphnids, likewise, the chromosome numbers appear to be the same in all types of female. Any chromosome difference which may conceivably be responsible for changes in the life cycle of these groups must therefore be invisible ones, and, in the aphids and cladocera, must be more or less easily reversible.

In the absence of a difference in the number of chromosomes between parthenogenetic and sexual females in rotifers, Storch attempted to push back the distinction to an early stage of development. He found that in *Asplanchna priodonta* the chromosomes in the early oocytes of a sexual female undergo synapsis and form tetrads, while the oocyte nuclei of the parthenogenetic female remain in a resting condition during the corresponding stages. These differences were to have been expected. He also found in the early oocytes of the parthenogenetic females certain highly refractive chromatic bodies adhering to the outer surface of the nuclear membrane, which were uncommon or lacking in the sexual females. Storch conceives these bodies to be of importance, perhaps organ-forming substances prepared in the nucleus and conveyed to the cytoplasm. It would be

easy to erect about these "Membrankörper" an impos-
ing edifice of theory. Those by whom mitochondria are
held in high esteem will no doubt gather these new ob-
jects into their fold, especially if they should prove to be
important. I refrain from suggesting the inhibiting or
stimulating influences which they could be imagined to
have upon various physiological processes, for these sug-
gestions will come in due time—and if not, so much the
better. To me, the "Membrankörper" appear rather to
be signs of a physiological difference not unconnected
with the greater growth of the oocyte in whose early
stages the bodies are present.

The importance of at least one of these early distinc-
tions between parthenogenetic and sexual females in
Asplanchna is overestimated by Storch when in his pre-
liminary account (Storch, 1923) he points out that what-
ever factors convert a parthenogenetic female into a sex-
ual one directly influence the chromosomes so as to bring
about synapsis—a statement which ignores the discovery
that in *Hydatina* the distinction between sexual and
parthenogenetic females dates from a much earlier stage.
The decisive event in *Hydatina* occurs in the last two or
three hours of the maturation of the parthenogenetic egg.
Influences known to be capable of converting a sexual
female into a parthenogenetic one are ineffective before
and after that time. If *Asplanchna* resembles *Hydatina*
in these respects, when an egg is laid the nature of the
female which will hatch from it has already been fixed.
The synapsis of the chromosomes and the formation of
the highly refractive membrane-bodies in the oocytes are
but results of determining events which occurred much
earlier. They may be used as recognition marks, or as
steps by which the different reproductive processes of the
adult are attained, but nothing more.

Failure of visible morphological entities to throw light
upon the nature of the cycle in rotifers, aphids and daph-
nians has driven some biologists to look for the necessary
determinations in the invisible phenomena of metabolism,

as is done also for the sexes of other animals, thus reviving a theory of an earlier age. Proponents of the theory referred to, pointing out that males and their spermatozoa are more active than females and their eggs, hold that anything which increases the rate of metabolism (by which is commonly meant rate of oxidation) tends to the production of males. As applied to the cycles which we are discussing, the theory would doubtless require that anything which increases the rate of metabolism tends to bring on the sexual phase, since in rotifers that is the only male-determination that exists. There are many facts that can be adduced in support of this view. As stated above, the sexual phase is regularly preceded and accompanied in rotifers by rapid growth, rapid reproduction, and large families, although Banta and Brown (1924) have found in cladocera that males are produced by a moderate reduction of the metabolic rate of the mother. The fact that the sexual phase in rotifers is hastened or rendered more pronounced by green food and probably by oxygen lends further support to the theory. Most of the artificial agents which have been used to modify the cycles of aphids and cladocera can be interpreted to act in like manner. What should be our attitude toward this theory?

Probably all biologists recognize that male animals are physiologically different from females of the same species. Probably all will accept the view that differentiation of the sexes during embryonic development is conditioned by differences in the metabolism of the two kinds of embryos. If it is asserted that these physiological differences may result from chromosome differences, there will be few dissenters. The statement that only chromosomes can account for such metabolic differences would be denied by some, perhaps by many. Announcement of the discovery of the particular metabolic difference in the embryos which causes the differentiation of the sexes is met with some skepticism. Even after it is shown that the oxidative processes of one sex are greater, or the stor-

age processes less, than those of the other, it has not been
shown that any means whatsoever of altering the rates
of these processes will alter sex differentiation. That
artificial alteration of the rates of these phases of metab-
olism may alter the developmental processes in the man-
ner required to distinguish the sexes remains an assump-
tion with but slight foundation. Oxidation and storage
are late enough in the chain of chemical events making
up metabolism to permit, or even to create, the hypothesis
that they are antedated by something else, and that they
may be results rather than causes. The hope that a sec-
ondary means of increasing oxidation, without going
back to its natural cause, would nevertheless alter sex is,
in my opinion, as futile as the belief that the speed of
lumber-production in a logging camp, though ordinarily
measured by the number of trees that fall, could be as
effectively increased by a tornado passing through the
forest as by sharpening the cutters' tools or by stimu-
lating the laborers to more vigorous operations. If, as is
not inconceivable, the changes from parthenogenesis to
bisexual reproduction and back again are closely allied
to the determination of sex, and if it is sought to apply
to these cycles the theory of levels of metabolism, the
same objections must be urged against regarding mere
rate of metabolism as the cause.

The expression ''metabolic level'' applied to the causa-
tion of sex or of cyclical changes in the mode of repro-
duction may be well enough chosen if the difference be-
tween maleness and femaleness, or between partheno-
genesis and bisexual reproduction, is purely quantitative,
even if it should prove that oxidation and storage are not
the phenomena whose level is at issue. But is the differ-
ence purely quantitative? Intersexuality and such phe-
nomena as the graded series of pigeon eggs obtained by
Riddle, the varying extent of periods of sexual reproduc-
tion in cladocera, and the probable gradual change in
aphids leading to the sexual period, seem to speak in
favor of a quantitative explanation. If these phenomena

are quantitatively determined, what then is to be said of the very sharp and definite differentiation in rotifers between the male-producing and female-producing types of female, which occurs almost momentarily in the maturation of the eggs from which these females develop? There are no intersexes in *Hydatina,* and no hesitation, so to speak, whether a male-producer or a female-producer shall develop. *Hydatina* is equally unequivocal in other matters; witness the considerable constancy in the number of cells in the adult body. There are other animals whose sex phenomena are as little subject to irregularity as are those of rotifers; but in *Hydatina* this decisive determination occurs in the midst of flux and response to the environment as great as that of any other organisms. The two conditions can be reconciled, it seems to me, only by assuming that the decisive event distinguishing parthenogenetic from sexual females is of a different order from metabolic level, but may be partly controlled by metabolic differences. In *Hydatina* this event is single and for some time irreversible. Animals with intermediate sex phenomena may be brought under the same point of view either by assuming several deciding events, or their reversibility, or both. Differences of this sort between animals appear to me much more likely than that their sex phenomena are essentially unlike.

If there is something more fundamental lying beneath the oxidation and storage levels that distinguish males from females, or sexual reproduction from parthenogenetic, what may this something be? Foolish questions invite, and sometimes receive, foolish answers, but the risk seldom prevents the asking. It should be particularly easy in this instance to provide the foolish answer, because, in our present state of knowledge, any answer will be regarded as foolish by some one. It were the part of wisdom to maintain silence, but playing at wisdom seldom makes progress, and it should be no more reprehensible to formulate hypotheses in public than to do so in the recesses of one's private laboratory. Whatever pos-

sible explanations are suggested will be derived chiefly from the evidence afforded by cyclical animals, and will be intended to apply only to them.

It is clear that whatever introduces the sexual phase in the aphids, cladocera and rotifers must be rather readily influenced by the environment. It is equally certain in the case of the rotifers that the determination is a sharply defined event, occurring in a very short time, perhaps momentarily, and maintaining complete irreversibility for a whole generation. From comparatively recent work on the manipulation of chromosomes by environment in other animals, it might seem that the most plausible explanation meeting these requirements would be one involving chromosomes. As pointed out earlier, no difference in the number of chromosomes between parthenogenetic and sexual females has been discovered in any of the groups exhibiting cycles of reproduction. It is conceivable, of course, that such differences may involve only behavior of the chromosomes, not their number, thereby implying a difference in chemical or physical constitution. Such a difference in behavior has been found by Morgan (1909) in certain phylloxera, where chromosome adhesions appear to be associated with the distinction between male-producing and female-producing lines. Adhesion of chromosomes may be due to changes in their viscosity, and our knowledge of viscosity of protoplasm is only in its beginnings. There is a great and rather sudden increase in the viscosity of the oocytes of the rotifers at the beginning of the maturation division, which is very nearly the time of the event which determines which sort of female the egg shall become. Whether the viscosity of the chromosomes is likewise altered at the same time, or whether it may be unequally altered in different oocytes, in response to quality of food or the presence of chemical substances in the water, is unknown.

If chromosome behavior in response to physical or chemical phenomena is not responsible for the changes in

the cycle, it is not inconceivable that there is a more direct or more general action of physical or chemical changes. Here a wide field of speculation is opened. When the subtle influence exerted upon higher animals by green or raw vegetable foods is thoroughly understood, it may be possible to conjecture why green protozoan food produces such a remarkable increase in sexual reproduction in rotifers. When it is known why unobstructed sunlight is so effective in stimulating certain phases of metabolism and preventing deficiency diseases, or why mere exposure of otherwise non-stimulating foods to sunlight has a similar effect, we may be nearer an understanding of sex and life cycles. It is a permissible conjecture that these influences are at least as much physical as chemical. It is not necessary to pursue the physical explanation of life processes as far as does Mathews (1924) when he adopts the view that living matter differs from non-living in the magnitude of its electronic orbits and the consequent greater energy involved. One who lingers over Mathews' engaging hypothesis is apt to imagine himself an electron, revolving about a nucleus of truth, though perhaps in ever-widening orbits until the energy involved is positively dangerous. We may perhaps wait until physicists have had an opportunity to express their judgment upon this particular hypothesis. Should they reject it, there is nevertheless room to anticipate, or at least to hope, that the metabolic process at the basis of oxidative levels, sex differentiation and modes of reproduction may nevertheless, if the search must go so far, some day be found to rest upon the ultimate structure of matter and energy.

LITERATURE CITED

Banta, A. M.
> 1918. Sex and sex intergrades in Cladocera. *Proc. Nat. Acad. Sci.* 4: 373–379.
> 1921. Selection in Cladocera. *Anat. Rec.* 20: 212.

Banta, A. M., and L. A. Brown.
> 1924. Rate of metabolism and sex control in Cladocera. *Anat. Rec.* 29: 147.

Davidson, J.

1924. Factors which influence the appearance of the sexes in plant lice. *Science* 59: 364.

Green, W. R.

1919. Studies in the life cycle of Simocephalus vetulus. *Biol. Bull.* 37: 49–95.

Hopkins, H. S.

1921. The conditions for conjugation in diverse races of Paramecium. *Jour. Exp. Zool.* 34: 339–384.

La Vaulx, R. de

1919. L'intersexualité chez un Crustacé Cladocère: Daphne atkinsoni Baird. *Comp. Rend. Acad. Sci. Paris* 169: 97–99.

1922. Sur l'apparition d'intersexués dans une lignée de Daphnia magna (Crustacé cladocère) et sur le déterminisme probable du phénomène. *Comp. Rend. Acad. Sci. Paris* 174: 1740–1742.

Marcovitch, S.

1923. Plant lice and light exposure. *Science* 58: 537–538.

Mathews, A. P.

1924. Some general aspects of the chemistry of cells. ''General Cytology,'' edited by E. V. Cowdry, Sec. II, pp. 15–95. Univ. of Chicago Press.

Morgan, T. H.

1909. A biological and cytological study of sex determination in phylloxerans and aphids. *Jour. Exp. Zool.* 7: 239–352. 1 pl.

Patch, E. M.

1920. The life cycle of aphids and coccids. *Ann. Ent. Soc. Amer.* 13: 156–167.

Shull, A. F.

1915. Periodicity in the production of males in Hydatina senta. *Biol. Bull.* 28: 187–197.

1918a. Relative effectiveness of food, oxygen and other substances in causing or preventing male-production in Hydatina. *Jour. Exp. Zool.* 26: 521–544.

1918b. Genetic relations of the winged and wingless forms to each other and to the sexes in the aphid Macrosiphum solanifolii. AMER. NAT. 52: 507–520.

1921. Chromosomes and the life cycle of Hydatina senta. *Biol. Bull.* 41: 55–61.

Shull, A. F., and Sonia Ladoff

1916. Factors affecting male-production in Hydatina. *Jour. Exp. Zool.* 21: 127–161.

Storch, O.

1923. Parthenogenese und Eireifung der heterogonen Rädertiere. *Zeit. f. indukt. Abst. Vererb.* 30: 309–312.

1924. Die Eizellen der heterogonen Rädertiere. *Zool. Jahrb., Abt. Anat. u. Ont.* 45: 309–404. 4 pl.

Tauson, A.

1924. Die Reifungsprozess der parthenogenetischen Eier von Asplanchna intermedia Huds. *Zeit. f. Zell. u. Geweb.* 1: 57–84. 4 pl.

Wesenberg-Lund, C.

1923. Contributions to the biology of the Rotifera. I. The males of
the Rotifera. Mem. Acad. Roy. Sci. Lett. Danemark, Copen-
hagen. Sec. Sci., Series 8, 4: 191–345. 15 pl.

Whitney, D. D.
1917. Relative influence of food and oxygen in controlling sex in roti-
fers. *Jour. Exp. Zool.* 24: 101–138.
1919. The ineffectiveness of oxygen as a factor in causing male-
production in Hydatina senta. *Jour. Exp. Zool.* 28: 469–492.
1924. The chromosome cycle in the rotifer Asplanchna intermedia.
Anat. Rec. 29: 107.

SEX DIFFERENTIATION IN THE BULLFROG
(RANA CATESBEIANA)

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THE problem of sex determination and differentiation
in animals is so complicated, the field so vast and the
opinions expressed on the matter so conflicting, it would
be impossible in the brief time at my disposal to attempt
a comprehensive discussion of this fascinating and diffi-
cult subject. Instead I shall confine my remarks to an
account of an embryological and cytological study of sex
differentiation in one lowly member of the vertebrate
group—the bullfrog. In the first portion of this paper
the facts of sex differentiation in *Rana catesbeiana* will
be presented, the latter part will be devoted to general
theoretical considerations.[1]

During the past six years study of *Rana catesbeiana*
material from various parts of the United States has
shown the existence of local races which differ markedly
in regard to the time of occurrence and character of the
developmental process by which the definitive testes of
the male animals arise. The male larvae of most bullfrog

[1] In this paper only the writer's views on sex differentiation in anurans
have been presented, owing to time and space limitations. Views differing
from those presented here will be found in the following references: Hert-
wig, R., Biologischen Centralblatt, 1912, Bd. 32; Kuschakewitsch, S.,
Festschr. f. R. Hertwig, 1910, and Witschi, E., Arch. f. Mikr. Anat., 1914,
Bd. 85 and 86; also Zeitschr. f. ind. Abst. u.Vererbgsl., Bd. 29.

races present an interesting and complex series of cyclical changes in gonad and germ-cell development, which may be lacking in other races inhabiting the same region. In some strains the definitive male gonad is developed very early in larval life and the two sexes are easily distinguished. These are called differentiated races, *i.e.,* there is no question as to the sex of the gonads, even in young tadpoles. Other local races show a peculiar gonadic development, chiefly affecting the males, the definitive testis sometimes not appearing until near the end of the second year of larval life. In the young larvae of such races the sexes can not be distinguished with certainty. Races exhibiting the peculiar development of the male gonads are called undifferentiated, that is to say, the morphological features of the definitive testis are not established until late, and in some races not until two years after the time when the definitive testis has formed in other races.

There is nothing noteworthy about the gonads of the differentiated races; at any time during development the sexes are easily distinguished. The situation is quite otherwise in the undifferentiated strains, for when the gonads of the larvae of such races are examined, say at metamorphosis, they may be grouped into three distinct types, testes, ovaries and a third type of gland not so easy to classify (Fig. 1). It is an elongate, cylindrical body, generally smooth in outline. This type of gonad differs very greatly from both testis and ovary and in some respects is intermediate between the two. The gonad resembles the ovary in regard to length and in the absence of sex cords and rete apparatus, but it resembles the testis in that it is cylindrical, generally smooth and attached to the fat body (Fig. 1, B and C). All gonads of this type ultimately transform into typical testes by a peculiar indirect method; in fact, all male gonads of the so-called undifferentiated strains first develop the peculiar type of gonad shown in B and C, Fig. 1. In some animals even after two years one may find a typical testis
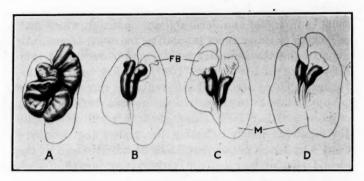
FIGURE 1

A. Ovary of a tadpole approaching metamorphosis; B, progonad of male
tadpole of same race and stage of development as A; C, progonad of larva
at metamorphosis transforming into the definitive type of testis shown in
D.   FB, fat body; m. mesonephros.

on one side and the elongated cylindrical gonad on the
other (Fig. 2).

According to some investigators the elongated, cylin-
drical gonads are really ovaries, and all the tadpoles of
the undifferentiated races such as here described are said
to be females, and later, under the influence of environ-
mental factors, 50 per cent. of the animals transform into
males.   The 50 per cent. which are thought to undergo
such a transformation are those larvae which possess the
peculiar gonads.   The writer does not regard this cylin-
drical type of gonad exhibited by males of the undiffer-
entiated races as an ovary, but rather as a sexually neu-
tral germ gland or progonad with neither sex predomi-
nating.   The germ cells may differentiate as spermato-
cytes, a few oocyte-like cells may appear or development
may proceed only as far as the pachytene stage and then
cease.   The sex-differentiating substances are evidently
present in the larvae in amounts sufficient to counterbal-
ance one another.   These progonads ultimately develop
into definitive testes by a remarkable transformation
process to be described later.

One very interesting point brought out in this study was the fact that differentiated and undifferentiated frog races may inhabit the same region or even the same pool. It is to be understood that the larvae of both types of races are similar in all respects except in regard to gonad development. ,
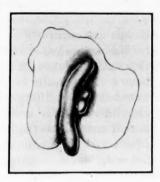

FIGURE 2

Gonads of larva at metamorphosis. At the left is shown the persisting progonad while on the right is the definitive testis. The transformation of the progonad into the definitive testis generally occurs more rapidly on one side than on the other in *Rana catesbeiana*.

But what is the explanation for the existence of differentiated and undifferentiated frog races? Why is it that in differentiated strains the ovaries and testes of the larvae are morphologically differentiated at a very early stage, whereas in undifferentiated races the gonads of the male animals undergo a series of peculiar changes, first developing a sexually neutral progonad, which is finally replaced by the definitive testis. Although the complete solution of the problem is not now at hand, the direction in which it is most likely to be found was clearly pointed out years ago by Professor Richard Hertwig in his interesting and suggestive hybridization experiments with *Rana esculenta,* where he was able to show unequivocally that the gonadic differences between the various local races of Central European frogs are due to differences in the hereditary constitution of the races.

Hertwig found that when males and females of differentiated races are bred, only morphologically sexually differentiated progeny result, and the sex ratio is 50 ♀ ÷ 50 ♂ ; on the other hand, when males and females of undifferentiated races are bred, only larvae showing indirect gonad development arise, or, according to Hertwig, who considered the elongate cylindrical gonad as an ovary, the F_1 generation are all hermaphrodites, in the sense that the animals are regarded as females, 50 per cent. of which later become males, or else sexually indifferent larvae, which were also thought to show first certain female characters. The results of crossing individuals of the differentiated and undifferentiated races gave results of particular interest. One and the same female crossed with different males gave different results, as also did crosses of one and the same male with different females. The following table summarizes several of Hertwig's crosses:

TABLE I

1. $\dfrac{♀ \text{ A} \times \text{a } ♂}{1 ♀ \div 64 \text{ J} \div 3 ♂}$ 2. $\dfrac{♀ \text{ A} \times \text{b } ♂}{69 \text{ J} \div 54 ♂}$ 3. $\dfrac{♀ \text{ A} \times \text{d } ♂}{50 ♀ + 52 ♂}$

4. $\dfrac{♀ \text{ B} \times ♂ \text{ a}}{109 \text{ J}}$ 5. $\dfrac{♀ \text{ B} \times ♂ \text{ b}}{34 ♀ \div 52 ♂}$ 6. $\dfrac{♀ \text{ B} \times \text{d } ♂}{142 ♀ + 140 ♂}$

7. $\dfrac{♀ \text{ L} \times ♂ \text{ f}}{52 ♂ \div 52 ♀ \div 2 \text{ J}}$ 8. $\dfrac{♀ \text{ L} \times ♂ \text{ 1}}{111 ♂ \div 101 ♀}$ 9. $\dfrac{♀ \text{ L} \times ♂ \text{ d}'}{3 ♂ \div 11 ♀ + 190 \text{J}}$

Undifferentiated Race a and d′	Differentiated Race b, d, 1, and f	J = Sexually indifferent animals, *i.e.*, morphologically sexually undifferentiated. A normal sex ratio becomes established some time after metamorphosis.

In the table males and females belonging to the same type, *i.e.*, differentiated or undifferentiated races, are denoted by corresponding letters. The table shows that female A produces when bred with a male of its own race practically nothing but indifferent individuals, of which half later will develop the definitive male type of gonad

by the indirect method. Consequently, both animals belong to the undifferentiated races. With the male b the same female (A) produces early sexually differentiated males and numerous individuals with sexually indifferent germ glands (denoted by J) which later on become females. Male b in this cross belongs to the differentiated race; when mated with male d, however, the female A produces normally differentiated individuals.

Female B crossed with male d gives the same result as the female A crossed with this male, whereas the result of the cross with male b is quite different from that where female A was crossed with the same male. The results of crossing female L with males F, 1 and d' give somewhat different results. The hybridization data are not clear, but the essential point here is that the experiments show clearly the zygotic nature of the factors involved in gonad development and differentiation of the two types of frog races.

Embryological study of seven frog races from different parts of the United States shows that the developmental history of the male gonads of differentiated races is much the same, whereas great variation exists among the undifferentiated strains. Practically every race differs in some respects from the others. Thus a race of larvae obtained from near Lawrence, Kansas, differs from any other studied, in the precocity of the maturation cycle of the progonad germ cells. The progonad of immature tadpoles of 60 mm may be filled with spermatocytes, yet the glands exhibit none of the morphological features of the definitive testes and are later replaced by normal male gonads. A race obtained from Douglas County, Kansas, and one from West Haven, Connecticut, resembled one another, but both differ from other races in that the sex cords do not appear until near the end of the second year of larval life and consequently the definitive testis which depends for its development upon the sex cords does not replace the progonad until metamorphosis or after. In most frog strains sex cord formation is not delayed two

years. A race found at Seymour, Connecticut, does not even present any morphological indications of sex differentiation until the tadpoles are about eight months of age. The germ glands remain mere undifferentiated clusters of germ cells long after sex differentiation has occurred in other races inhabiting the same region.

Because of these facts, it is necessary, in any attempt to explain the peculiar gonadic condition of frogs, to assume that besides the fundamental zygotic difference in the factors for gonad formation between the two types of frog races, the undifferentiated races vary among themselves slightly with regard to the potency or intensity, say, of the factors concerned with gonad differentiation. Hence, when such races are crossed, and they intermingle freely in nature, all kinds of qualitative and quantitative variations in gonad development and differentiation appear.

However, to say that the cause of the peculiar and variable gonadic conditions exhibited by the local frog races is zygotic in origin does not solve the problem. We are still confronted by the question as to the significance of the germ gland and germ cell changes of the undifferentiated strains. The essential problem facing the student of sexual conditions in tadpoles is the determination, beyond peradventure, of the nature of such glands as those shown in Fig. 1, B and C. Are such gonads male, female, sexually neutral or intersexual, and if the latter, to what degree? Statistical study of the sex ratios and hybridization experiments throw no light upon this problem, and our only recourse is to embryology and cytology. The developmental history of germ cells and germ glands of the various races should give some clue to the solution of the problem. We shall begin with a brief description of the morphologically undifferentiated germ gland common to all races in early larval life.

If the gonads of 25–30 mm tadpoles are examined they will be found to consist of a peripheral ring of large germ cells, enclosing a central cavity filled with web-like con-

nective tissue. The cavity is the primary genital space and the germ cell ring is the germinal epithelium. Segmentally arranged masses of cells from the mesonephros project into the gonad and develop large cavities. These structures are the sex cords and the sex cord cavity is the secondary genital space. The sections through this type of gonad shown in Figs. 3, 4 and 5 are characteristic of all bullfrog tadpoles at an early stage and exhibit the ground plan of gonad structure out of which both ovary and testes are differentiated.

The ovary represents a further development of the structurally indifferent gonad. If such glands as shown in Figs. 3, 4 and 5 become ovaries the sex cords cease growth and the germ cells of the epithelium increase greatly in number. The sex elements arrange themselves in "nests" or clusters surrounded by mesenchymal cells. The cells within the nests or cysts are sister cells and pass through their cyclical changes simultaneously.

The chromosomes of the germ cells of female larvae very early undergo synapsis and the cells enter the growth period, pass through the early stage of pseudo-reduction and become oocytes. The germ cell nests or cysts are destroyed by the enlarging cells and only a few persist at the periphery of the gonad (Fig. 6).

The sex cords form in the young ovary just as in the testis but do not develop very far and are abortive structures. They give rise to the rete ovarii and the ovarial sacs.

In contrast to the ovary, the site of testis formation in differentiated strains is shifted from the peripheral germ epithelium to the sex cord region and any tadpole is to be regarded as male whose sex cords show the presence of germ cells. The sex cords of male larvae at the time of sex differentiation are structurally identical with those of the ovary and undifferentiated gonad. However, they show great activity in that cells migrate out from them to the germ cells of the germinal epithelium, thus forming short cross cords with expanded ends (Figs. 7 and
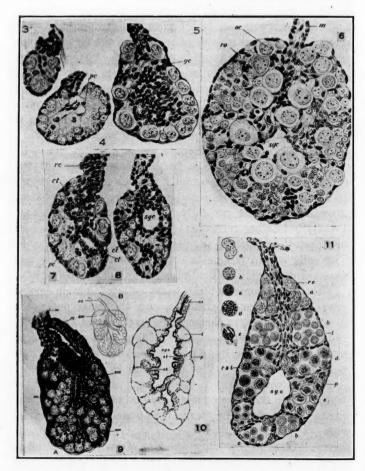
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FIGURE 3

Section through the undifferentiated gonad of a 25 mm tadpole. The small cells at the hilus region are migrating sex cord elements.

FIGURE 4

Section through the gonad of a 30 mm tadpole between the segmentally arranged sex cords. pc is the primary genital space.

FIGURE 5

Section through the sex cord region of an undifferentiated gonad at its maximum development. gc, germinal epithelium. The mass of small cells constitute the sex cords.

8, CT). These grow around the sex cells and the germ elements pass into the walls of the sex cords. The latter grow very rapidly and anastomose with one another, forming a core of tissue extending the length of the gonad. All the sex cells pass into the cords in the manner described, leaving the peritoneum empty. After reaching the sex cords the germ cells multiply rapidly and form solid cyst-like structures surrounded by mesenchymal elements. These are the fundaments of the testis ampullae, which later by elongation become the short sperm tubules and connect with the vasa efferentia (Fig. 9, am). The essential features of testis differentiation are established with the appearance of the ampullae, and we need not concern ourselves here with the later development.

The early stages of gonad differentiation in the future males of the undifferentiated races are similar to the ini-

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FIGURE 6

Section through a young ovary. Sgc is secondary genital cavity (sex cord cavity); oc, oocyte; ro, residual oogonium. Note that the sex cords of the undifferentiated gonad (Fig. 5) play no rôle in ovarian development.

FIGURES 7 AND 8

Sections through gonads showing the first signs of differentiation into testes. At CT are cords of cells from sex cords passing out to germ cells in the epithelium and the latter passing into the cords. Sgc, secondary genital space; pc, primary genital space.

FIGURE 9

Section through young testis. The germ cells have passed from the epithelium into the sex cords and are organized into clusters surrounded by mesenchymal elements. The definitive sex cords or rete cords, rc, have formed and are connecting up by short tubules with the germ cell clusters, or ampullae, am.

FIGURE 10

Section through the progonad of larvae of an undifferentiated race. Animal approaching metamorphosis. Note the greatly thickened walls of the gland divided into lobules, 1; the definitive sex cords, rc, are just appearing after two years. Cross tubules, ct, are growing out to the lobules.

FIGURE 11

Section through the progonad of a 60 mm larva before the definitive sex cords have formed. Note the lobules filled with spermatocytes and the migrating sex cord cells at rc. The residual spermatogonia are shown at rsl. A–e, indicate the type of maturation cells present.

tial steps in ovarian formation (Fig. 11). The single layer of germ cells in the epithelium thickens by several strata and the cells become arranged into clusters, each group surrounded by a mesenchymal covering. The sex cells, with the exception of one or two in each cyst, enter pseudoreduction, just as do those of the ovary, and pass through the early maturation stages. At this stage of development the gonad resembles the young ovary at the beginning of differentiation. Both are sacs of germ cells, the latter arranged into nests or cysts forming the thickened wall (so-called germinal epithelium) of the gonad surrounding a central cavity. However, there is one very important difference between germ-cell development of the ovary and that of the progonad of the males of the undifferentiated races. In the ovary the germ cell cysts are soon obliterated by growth of the oocytes (Fig. 6), whereas in the males of the undifferentiated races (Fig. 11) these cysts form the anlagen of sac-like lobules radially arranged about the central sex cord cavity which persist and form the progonad. Moreover, the germ cells of the ovary soon enter the second growth period, becoming typical ova. Conversely, in males of the undifferentiated races the progonad germ cells only rarely assume an oocyte-like character, but instead become spermatocytes or degenerate at some stage of the maturation cycle in prespermatogenesis. Compare Figs. 6 and 11.

The germ cells begin maturating soon after the formation of the lobules, and all stages of the germinal cycle up to the anaphase of the heterotypic division are encountered (Figs. 11 and 17–34). Oddly enough, the first maturation division is never completed. The spindle apparatus breaks down owing to fragmentation of the centrosome, each fragment sends out fine filaments which attach themselves to the tetrads and the latter are literally pulled to pieces. Sometimes giant spermatid-like structures are formed by growth of an axial fiber from the centrosome of a degenerating spermatocyte (Fig. 26). In some progonads, along with the abortive sperma-

tocytes, are large oocyte-like cells, and it is not at all uncommon to see the two types of cell side by side within the same lobule. Many gonads do not show the oocyte-like bodies, whereas in others they are fairly numerous (Fig. 30).

The condition just described where the progonad of the future males of the undifferentiated races consists of greatly thickened walls divided into numerous lobules, the cell contents of which are undergoing maturation and degeneration, persists in some races for two years; in others, for a much shorter period (Figs. 10 and 11). During this time no indications of definitive sex cords may appear, and consequently such progonads lack rete apparatus and efferent ducts. The appearance of the sex cords is sudden and marks the beginning of typical testis formation, for with the development of the cords the entire morphological character of the progonad undergoes a radical transformation.

The sex cords form from the extragenital sex cord cells just outside of the gland, as open tubules extending from the hilus, deep into the gonad, giving off side tubules which grow out to the lobules of the progonad which until this time were mere blind sacs of maturation cells (Fig. 10, rc and ct). These cross tubules from the main sex cord abut directly against the residual spermatogonia of each lobule, which have persisted unchanged through the maturation cycle (Fig. 11, rsl). Cells from the tubules then grow around these spermatogonia, cutting them off from the lobules, and they now pass into the sex cords (Fig. 12). Generally one residual cell is left in each lobule (Fig. 13, rsl). As development proceeds the sex cord walls become filled with the residual spermatogonia which have migrated down the cross tubules, and the ampullae of the definitive testes start developing. These structures arise in a peculiar manner: small areas of the sex cord wall, bearing one or more of the spermatogonia, bulge out into the sex cord cavity and growing from the base region are pushed across the cavity to meet and fuse
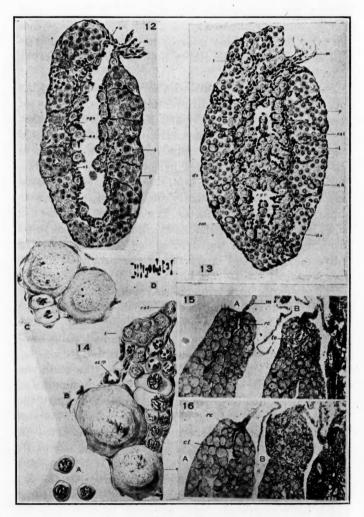
FIGURE 12

Section through the progonad of a larva showing the first stages in the formation of the definitive testis. The residual spermatogonia of the lobules have passed down the cross tubules, ct, into the sex cords, rc.

FIGURE 13

Advanced stage in formation of the definitive testis. The germ cells in the sex cords have multiplied and are arranged into clusters, the testis

with the opposite wall. The cavity (see Fig. 12) is eventually cut into very small compartments in this way, each surrounded by a ring of spermatogonia and representing the fundament of an ampulla or sperm tubule (Fig. 13, am).

The gonads at this stage show an outer husk or rind of progonad lobules filled with degenerating maturation cells and a central core of spermatogonia arranged into testis ampullae connected by tubules with the segmentally arranged vasa efferentia (Fig. 13). Eventually all the maturation cells of the progonad lobules degenerate, the lobule being slowly refilled with secondary spermatogonia arising from the residual cells remaining within the lobules. Such lobules may be directly converted into definitive testis ampullae, or they may be broken up and formed anew. There is some variation among the races in regard to this point.

Almost immediately upon completion of the definitive testis, a second prespermatogenic cycle occurs, but this time morphologically normal spermatozoa are produced.

ampullae, am. Note the outer rind of progonad lobules filled with maturation cells. Rsl, residual spermatogonia in lobule; ds, degenerating spermatocyte; nh, degenerating cells showing oviform hypertrophy—these cells are not oocytes.

FIGURE 14

A. Typical first spermatocyte prophase tetrads from gonad shown in figure 16A; B, enlarged drawing of a portion of the gonad shown in figure 16A. Note spermatocytes and oocyte-like cells side by side; C shows spermatocyte division figures together with oocyte-like cells; D, the tetrads shown on the spindle in C. All these drawings are from the gonad shown in figure 16A. Rsl, residual cell; 1, lobule; CT (?), probably the cut end of a cross tubule.

FIGURE 15

Either an ovary, or an organ of Bidder, transforming into a testis. A and B are gonads of the same animal; rc, sex cord; te, testis forming.

FIGURE 16

Sections through the same gonad as shown in figure 15. One gonad, A, shows first appearance of the sex cords and cross-tubles, ct; the other, B, shows the transformation of the hilus (sex cord) region into a testis.

It is to be emphasized here that the maturation cycle occurring in the progonad is abortive, mature sex products are never produced, and it is peculiar in other respects. The size of the germ cells and chromosomes is much greater than that characteristic of the maturation cycle of the definitive testis. Probably the most striking difference is the type of tetrad formed. The bivalent chromosomes of the progonad are strikingly similar to those of urodeles, being large open rings, whereas the bivalents of the definitive testis spermatocytes are small thick rings or dumb-bell-shaped bodies. I have never seen tetrads in adult frogs which resembled those of the larval progonad.

It is obvious that the process we have just described involves the formation of two gonads in males of the undifferentiated races, a progonad and a definitive testis, and that the germinal elements of the latter arise from the residual spermatogonia of the former (Figs. 12 and 13). The character of the progonad is not uniform, however, for in almost every undifferentiated race it differs in some way in regard to the degree of development and differentiation attained before being replaced by the definitive testis. It is highly developed in some races and persists for two years without any indication of retrogressive changes. In other strains it is replaced by the definitive testis early in larval life, and even in the same local race much individual variation occurs. The same thing is true with regard to the degree of sex differentiation exhibited by the progonad. In some races, notably one found at Seymour, Connecticut, the gonad is totally undifferentiated and exists as a mere cluster of indifferent germ cells until the animals are approximately eight months of age. In other strains, the progonad is filled with spermatocytes, and insofar as the cell cycle is concerned is male, but the gonad has none of the structural features of a frog testis and is destined to degenerate and be replaced by a definitive testis. Figure 11 is of this type of gland. On the other hand, the progonad of some

races may exhibit spermatocytes and oocyte-like cells side by side, or neither may appear, and only germ cells in early pseudo-reduction stages and residual spermatogonia are found.

The belief was expressed early in this paper that the peculiar gonadic cycle characteristic of the males of the undifferentiated frog strains is zygotic in origin, and that the minor variations in gonad development and differentiation occurring among the local undifferentiated frog races was due to hybridization of local races, each probably differing from others by minute quantitative variations in the amount of sex differentiating substances distributed by the chromosome mechanism at fertilization. However, the question of the significance of the germ gland and germ cell changes, from the standpoint of sex differentiation, was left unanswered pending the results of embryological and cytological study.
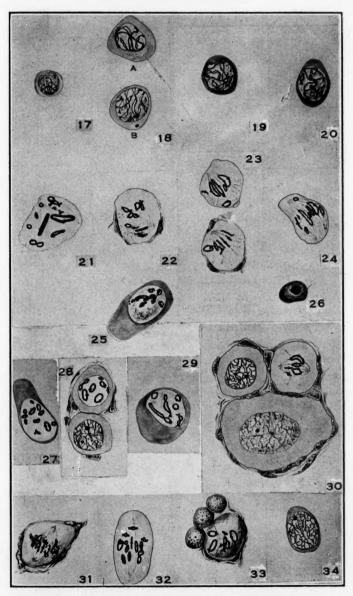
If we accept the view prevalent in some quarters that the elongate, cylindrical progonad is really an ovary, then the problem would be simplified and we could say that we were possibly dealing with a case of zygotic intersexuality comparable to that described by Goldschmidt for the gypsy moth. However, it is evident that the progonad is not an ovary; in fact, it differs from an ovary in several well-marked features (Fig. 11). The progonad germ cells appear to be sexually neutral and capable of differentiating either into oocyte-like cells or spermatocytes, or of remaining for long periods in the pachytene stage and finally disintegrating without developing either way. But no matter which line of sex differentiation the majority of the germ cells follow, the entire germ gland undergoes degeneration and is replaced by a different type of gonad—the definitive testis.

It is the writer's belief that the first formed germ gland of males of the undifferentiated frog races is really a sort of progonad which is sexually neuter, owing to the balance struck between the male and female sex-differentiating substances distributed to the zygote by the

chromosome mechanism. In these races, owing to the balanced condition of the contrary sex-differentiating substances in the larvae, the germ cells of the progonad can differentiate along either male or female lines, depending upon the quantitative relations existing between the sex-differentiating substances within the cells. In bullfrog larvae, in general, the male features predominate so that in many larvae the progonad can legitimately be called a protestis (Fig. 11).

The development of the progonad into a testis is hardly comparable to the transformation of an ovary into a testis, such as described for intersexual gypsy moths. In these forms the ovary develops in a perfectly normal fashion and ova and egg tubes are typical, then at a certain critical point, the "switch over" reaction occurs and the ovary disintegrates and a testis forms. However, the progonad of the tadpole is far from being an ovary, but is in fact a sexually neutral gonad which may persist for a few months or for two years, and present all kinds of quantitative variations among the local races as to the degree of development attained. The progonad is sexually neutral, not from any lack of male and female sex-differentiating substances, but because they are present in the larvae in practically equal quantities, and the germ gland is the morphological resultant of the balance struck between them.

If the sexual neutrality of the progonad is due to balancing of the contrary sex-differentiating substances resulting from the hereditary factors introduced into the zygote at fertilization, then it is to be expected that cases will occasionally arise where the equilibrium has been temporarily disturbed and one or the other sex gains the ascendency. Such cases have been encountered.

In the mass of material studied, four cases appeared which seemed beyond doubt to represent the actual transformation of an ovary into a testis, the ovary in these cases being progonads which greatly resembled a Bidder's organ such as occurs in Bufo. Microscopically the

FIGURES 17–34—The maturation cycle of the progonad germ cells.

gonads looked like ovaries or Bidder's organs and were filled with oocyte-like cells (Figs. 15 and 16). At the hilus region, however, definitive male sex cords were present with cross tubules growing out toward the peripherally located residual cells. The oocyte-like cells in the vicinity of the sex cords were degenerating and being replaced by typical testis ampullae and spermatogonia. In the regions between the sex cords the gonads could not be distinguished from true ovaries or Bidder's organs.

Close cytological study of the gonads revealed numerous cysts of prophase spermatocytes and cells at the anaphase of the heterotypic mitosis side by side with apparently typical oocytes (Fig. 14). Such spermatocytes did not appear in the testis portion of the gonad— only spermatogonia were present there—and they differentiated directly from the diplotene cells of the young lobules of the gonad. Their appearance coincided with the appearance of the sex cords. Thus the evidence is very strong that previous to formation of the sex cords the younger maturation cells at the periphery of the gonad developed into oocyte-like cells and not into spermatocytes. The sex cords apparently secreted or were associated with some substance, *i.e.*, enzyme or hormone which destroyed the large oocyte-like cells, stimulated multiplication of the residual cells and induced cells in the early stages of pseudoreduction to develop into spermatocytes. In these few cases the progonad appeared to be predominantly female. To offset such examples of the early dominance of the female sex-differentiating substances, we have numerous examples showing the dominance of male influences on the progonad, where the maturation cells all developed into spermatocytes, the definitive testis not appearing until the sex cords formed some time later (Fig. 11).

Throughout this paper constant reference has been made to the sex cords as highly important factors in gonad development and differentiation. This point can

not be too greatly emphasized, for the length of persistence and degree of development attained by the progonad of the various local frog races depends upon the time of appearance of the definitive sex cords. It is greatest in those races where the sex cords do not form until after two years, and least in the strains where the cords develop early in larval life. The fate of the progonad is sealed when the definitive sex cords form, for immediately changes occur which lead to establishment of the definitive testis and destruction of the progonad, no matter whether its germ cells are differentiating as oocyte-like elements or spermatocytes or neither. The sex cords apparently are the morphological expression of the active determining agents for definitive testis development and exert or are associated with some influence which enables them to override any differentiating tendencies the progonad and its germ cells may have exhibited previous to their formation.
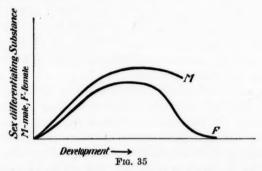
It will be recalled that sex cords are formed in the morphologically sexually indifferent gonad of bullfrog larvae of all races (Figs. 3, 4 and 5). In females of differentiated races and in all animals of the undifferentiated strains, the only trace of sex cords the gonads possess are those of the indifferent gland. The sex cords of males of the differentiated races grow rapidly and become very active, sending out side growths which pass around the sex cells of the germ epithelium and literally drag them into the cords, so to speak (Figs. 7 and 8). The second formation of cords, *i.e.*, the active definitive structures of males of the undifferentiated races, does not appear until late; consequently the gonad which continues development in their absence lacks the necessary morphological basis for definitive testis formation and forms a hollow sac of lobules and remains so until the sex cords appear (Figs. 10, 11 and 12). The definitive testes can not develop in the absence of sex cords and the appearance of the latter definitely decides the course of development. Since the sex cords are peculiarly male

structures developed under the stimulus of male sex-differentiating substances, and since they so profoundly transform the morphological features of the progonad built up in their absence, it seems not unlikely that they secrete or give rise to some physiological agent, probably of hormonal or enzymic nature, which induces the radical changes leading to definitive testis formation. The time of appearance of the cords in males of undifferentiated races is probably zygotically determined, though what the factors are that delays their formation is unknown.
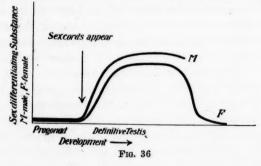
Up to the time of appearance of the definitive sex cords the progonad is sexually neutral, the germ cells apparently possessing the power to differentiate either into spermatocytes or oocyte-like elements or of remaining in early stages of pseudoreduction and not differentiating either way. Following sex cord formation the sexual neutrality of the progonad no longer exists and the residual spermatogonia of the old gonad become the germinal elements of the new testes—the maturating cells degenerating no matter whether they are oocyte-like elements, first spermatocytes or cells in early pseudoreduction stages showing no differentiation (Figs. 10, 11, 12, 13 and 15).

Our study of sex differentiation in *Rana catesbeiana* leads to the conclusion that in frogs the sex-determining and differentiating factors are distributed by means of the chromosome mechanism, and that there are two opposing sets of factors, one of which (that for maleness, since the male frog is probably heterozygous for sex) is carried elsewhere than in the sex chromosome, probably by the autosomes, as suggested by the work of Bridges on Drosophila. It is necessary to assume that factors for maleness and for femaleness are present in every fertilized egg or for that matter in every germ cell of the resulting individual in a definite quantitative relation, the given sex of both individual and cell being the resultant of the proportional relation existing between these quantitative determiners.

In the differentiated frog races from the beginning of development the quantitative relations of the male and female sex-differentiating substances distributed to the zygote by the chromosomes are such that the sexes are clearly differentiated at an early larval stage. The idea may be graphically represented by modifying the scheme employed by Goldschmidt to explain his intersexual moths.

Fig. 35

On the other hand, the quantitative relations of the sex-differentiating substances in males of the undifferentiated races are such in early larval life that the developing gonad is neither a definite testis nor an ovary, but a peculiar sexually neutral structure, combining in a curious fashion the morphological features of both ovary and testis, and from the standpoint of differentiation poised uncertainly between the two sexes but not differentiating definitely into either. The idea may be expressed thus:

Fig. 36

The balanced condition of the contrary sex-differentiating substances of the progonad is definitely overthrown with the appearance of the sex cords. These structures clearly exert a decisive influence, bringing about degeneration of the progonad and formation of the definitive testis from its residual cells, but the physiological nature of the agents through which the cords work is unknown. It is obvious that the cords themselves are but the morphological expression of deep seated physiological changes occurring in the organism.

Since the sex ratio of adult bullfrogs of all races is 50–50, and the elongate, cylindrical progonads invariably develop into definitive testes, the 50 per cent. of animals of the undifferentiated races possessing these glands are to be regarded as genetic males exhibiting in the course of larval development a transitory, sexually neutral phase. In large numbers of these larvae, however, the progonad is not neutral but is really a protestis in that the germ cell cycle is distinctly male (Fig. 11).

The process by which the progonad is transformed into a definitive testis is not to be regarded as sex reversal (except possibly in a few exceptional cases, and even here it is impossible to know whether one is dealing with a true ovary or a transitory gonadic rudiment comparable to Bidder's organ in Bufo) because the progonads are not ovaries and sex is not really established. Indeed, even in the numerous cases where the progonad shows great predominance of male sex-differentiating influences (Fig. 11), and could legitimately be regarded as a testis, the developmental cycle remains unchanged, the sex cords appear, and the first-formed gonad gives place to a definitive testis.

SEX DETERMINATION AND SEX DIFFERENTIA-
TION IN BIRDS AND MAMMALS

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IN a brief review of this broad and intricate subject with its many points of debate, and even dispute, it will be necessary to some extent both to deal in generalities and to handle the subject in a somewhat arbitrary and at times in a dogmatic fashion.

SEX DETERMINATION

Briefly stated, our present knowledge leads us to believe that sex in birds and mammals is determined at fertilization. Some would associate sex determiners with chromosomes, others with differences in metabolic rate, but it seems possible, even probable, perhaps, that the two conceptions are really but different indicators of the same basic physiological condition and that we will find no contradiction when dealing with the two ideas.

Investigations of mammalian chromosomes have revealed in general a specific male and female chromosome constitution—an XX female condition, an XY male type, though some would deny the Y element in the male. In any scheme the male from a morphological aspect would be the heterozygous individual and genetic evidence bears out the supposition. The most modern chromosomal analyses of Painter ('22, '23, '24) on the opossum, monkey, man and horse would indicate such a condition to hold for mammals in general. Thus by segregation of the male XY elements and recombinations with the segregated XX female elements we have a morphological association of chromosomes with sex that indicates sex determination at fertilization or zygotic sex determination.

In the bird genetic evidence has for a long time led to the supposition that the female instead of the male is heterozygous for sex; theoretically the ova of birds are

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of two types, whereas all spermatozoa are alike. Within the last year or two the work of Stephens, Hance ('24) and of the Russian zoologist Shiwago ('24) demonstrates conclusively the ZZ male constitution and the WZ female type; thus genetic evidence for female heterozygosity is confirmed by morphological data. Riddle ('17) has given us a physiological demonstration of two classes of ova— a high storage, low metabolic type developing into females and a lower storage capacity, higher metabolic rate eggs producing males. Both the morphological and physiological evidence confirms the earlier genetic evidence and leaves little doubt that sex is determined at fertilization.

Granting, however, the zygotic determination of sex we are faced with graded expressions in differentiation (see Lillie '17b). Sex is coming to be recognized as having a quantitative as well as a qualitative aspect, and the expressions of latent possibilities of both sexes are often encountered in which elements of the two different sexes have developed simultaneously. Ordinarily, there is a normal balance of sex determiners, whatever their nature, that lead to a more or less pure expression of primary sex differentiation; but an occasional disturbance of such a balance often allows characters of the recessive sex to appear. We should bear in mind, therefore, the distinctness of sex determination and sex differentiation. Primary sex determination in birds and mammals is usually followed by the development of a typical male or female. The zygotic sex-determining factors alone, however, appear to be limited in their capacities as differentiators of sex and a second and supplementary factor, which may be termed the hormonal factor, is recognized as of great importance. This factor is commonly considered to be elaborated as a chemical substance or substances in the sex gland and distributed through the blood stream; but it is thoroughly appreciated that its elaboration, at least its expression, is dependent upon a balanced physico-chemical relationship to some extent influenced by other organs of internal secretion.

Sex Differentiation in Birds

Examples of the two influences, primary zygotic factors and hormonal factors, are beautifully illustrated in sex differentiation in the bird. Specifically, in the Brown Leghorn breed of the domestic fowl, the cock, aside from the characteristic head furnishings (comb and wattles), spurs and psychic tendencies, possesses an outstanding feather pattern not only in feather form but in its brilliant coloration. The female, on the other hand, differs in head furnishings, absence of spurs and both feather form and color. To determine the value of the hormonal influence in this marked sex differentiation it is necessary to eliminate it so far as possible by gonad removal. If this is done sufficiently early we have to some extent a true expression of the potentialities of the zygotic sex factors as sex differentiators. So far, however, gonad removal has not been accomplished until after hatching and it is difficult to know to what extent these may have been active during embryonic life.

In the Leghorn cock early, and to some extent later, removal of the testis produces a capon differing primarily from a normal cock in the absence of sexual instincts, and smaller, less vascular head furnishings. The typical cock feathering remains unchanged in form and color, unless perhaps the length and brilliancy of feathers are slightly enhanced; spur growth is unaffected. Spurs and feather pattern, long considered dependent upon testicular secretions, are seen to be due not to hormonal influences but to be an expression of the zygotic factors in differentiation. From these results, therefore, we may conclude that the supplemental or hormonal factor in male sex differentiation conditions the psychic nature, the head furnishings and perhaps to some extent body proportions.

We have been inclined to think that hen feathering was due to the lack of testicular secretions; but removal of the ovary has changed these suppositions. Following ovariotomy spurs begin to grow; growing feathers are

changed in form from the simple hen type to the long feathers of the cock, and the sober color is replaced by the brilliancy of cock feathering; the head furnishings resemble those of the capon to a large extent. The end result of changes after ovariotomy and castration, in each case removing the hormonal factor in differentiation, is the production of two birds very similar in external form and behavior. This type has been characterized by Pezard ('24) as a "neutral form" and by Lipschutz ('19) as an "asexual form." These workers would, therefore, throw the entire burden of sex differentiation on the hormones, but it should be remembered that the very development of specific hormone-producing tissues is in itself an expression of primary sex differentiation. Primary sex differentiation is therefore dependent upon zygotic factors, whereas secondary sex characteristics are largely dependent upon hormones for their differentiation. It will be appreciated that, in the hen, the ovarian hormones supplement the zygotic determiners by stimulating the development of hen feathering, by suppressing spur growth, by regulating specifically the head furnishings and by stimulating the development of the psychic behavior.

It can be shown that the hormonal factor is more powerful than the zygotic factors in the differentiation of the outstanding secondary sex differences by implanting the gonads of one sex into the opposite sex. Thus we know from the work of Pezard, Sand and Caridroit ('24) and Zawadowsky ('22) that an ovary transplanted into a castrated cock will stimulate development of hen feathering with consequent elimination of the brilliant cock feathering. Experimental gynandromorphs have been produced by plucking feathers from one side of the male bird along with, or following, ovarian transplantation; new feather formation under the ovarian influences will be of the hen-feathered type, whereas the old, unplucked feathers are of the cock type; an ovarian graft in the castrated male will produce the female head furnishings. In some of

these radically influenced differentiations it is almost impossible to distinguish a modified cock from the hen, except perhaps for size, despite the male determination. The hormonal influences have so modified differentiation that apparently (though not really) the opposite sex has been developed. In no sense, however, are we justified in stating that sex has thus been completely reversed.

The opposite condition, or a masculinizing effect, may also be realized. An ovariotomized hen with a testis transplant may be apparently converted into a male bird with the feather form and color of the cock; with typical cock headfurnishings, spurs and to a large extent psychic impulses those of the cock.

All these modifications relate to differentiation of secondary sex characters and it appears that the hormonal factor has no influence on the primary sex characters or gonads *once these are differentiated.* Thus Pezard and others have shown that a functional testis and ovary may coexist without detriment or influence to either. In the broadest sense, therefore, a true hermaphroditic condition has been produced experimentally.

It can be shown that the primary sex characters (gonads and ducts) are capable of being influenced in their differentiation by hormonal agents if these act during embryonic development. Minoura ('21) has demonstrated that a gonad graft made on the chorio-allantoic membrane of a developing chick produces gonad and duct relationships that deviate widely from the normal. Thus introduction of testicular hormones into a developing female produces a modification of the ovary and Mullerian ducts to the extent that a reversal towards the male development is indicated; similarly ovarian hormones introduced into a developing male appear capable of influencing development in the female direction. There is not yet direct evidence that complete sex reversal can be brought about solely through the hormonal influence.

Recent reports of Riddle ('24) and Crew ('23), however, prove that a primarily sex-determined female may,

under the influence of pathological conditions, suffer a
complete reversal and become a male individual with
functional testes, due, as Riddle would believe, to a radi-
cally reversed metabolic condition.

That certain latent potentialities of the male sex exist
in a determined female (the so-called bisexual potentiali-
ties) has been shown recently in another way by Zawa-
dowsky ('22), Benoit ('23) and Domm ('24). Complete
removal of the persistent left ovary of the bird at an
early age is sometimes followed by an increased growth
of the undeveloped and normally non-functional right
gonad or tissue closely situated. In this development it
may produce a testis-like organ and in the most extreme
cases all testicular elements, including spermatozoa, are
found. This unexpected and phenomenal result is corre-
lated with a bird of typical cock feathering, head furnish-
ings, spurs and to some extent a male psychic nature;
on removal of the regenerated testis the bird reverts to
the capon type.

One may conclude, therefore: (1) that normally, zy-
gotic sex determiners are able to carry primary sex
differentiation to the stage of the production of a specific
hormone producing tissue; (2) that under pathological
conditions a differentiated ovary may subsequently be
replaced by a functional testis; (3) that under certain
conditions both types of gonads may be present simul-
taneously in the same individual as in hermaphrodites;
(4) that the normal female following early ovariotomy
may develop a testis capable of producing spermatozoa;
and (5) that hormones produced by the developed gonad
enhance sex differentiation by stimulating development
of the secondary sex characters; these latter, correlated
with the gonad, may be of male, female or mixed charac-
ter and being dependent upon the gonad present are like-
wise subject to reversals.

In the bird, therefore, we have recognized zygotic sex
determination, the bisexual potentialities and the influ-
ence of a secondary hormonal factor in sex differentia-
tion.

MAMMALS

In the mammal, if we can accept the zygotic determination of sex as conclusively demonstrated and turn to differentiation of sex, we have again the fundamental zygotic sex factors to balance against the hormonal ones, fully realizing that the hormonal ones are but an expression or more complete development of the zygotic sex determiners.

Our attention should again be directed to hormonal influences operating (1) during embryonic development, wherein the primary sex characters (gonads and ducts) are influenced, and (2) during postnatal differentiation, wherein we are restricted to the secondary sex characters. Normally the gonads and ducts develop accordingly as the individual is a determined male or female and any influence affecting the primary differentiation must act during embryonic life. Once structurally formed it appears that hormones are incapable of modifying the gonads despite the fact that some non-hormonal influences may affect them.

We are restricted to a single clearly analyzed case of hormone influence acting during development in the mammal—that of the free-martin studied by F. R. Lillie ('17a) and Tandler and Keller ('11). Due to peculiarities in membrane relations, calf twins become so connected through membrane anastomosis that the blood of the two individuals is mixed. Only in the case of differently sexed twins is there any sexual modification and here specifically the female member of the pair is influenced. The ovary of such an individual (called a free-martin) fails to develop as would be expected in a zygotic female and instead is modified decisively in the male direction. The gonad instead of being a typical ovary may be highly similar in structure to an undescended testis; uterine tubes are underdeveloped or absent, Wolffian ducts being preserved instead; seminal vesicles appear. Despite this extreme modification, however, there is no direct evidence that complete sex inversion is possible under the condi-

tions·stated. The modifying influence in this case is believed to be hormonal, that the hormone is produced in the testis of the male of the pair of twins and carried in the blood stream to the female member of the pair, thus influencing the development of the latter. It would thus appear that hormonal modification of a determined primary sex character is possible *during its formation,* but we are without substantial evidence that such is possible *after primary differentiation.*

Hermaphrodites in mammals (excluding the free-martin) are not known to be results of hormonal influences. Primarily the condition appears to be due to unbalanced zygotic factors with the appearance of both gonad types; subsequently, hormones from each may enhance the differentiation of secondary sex characters with the consequent appearance of maleness and femaleness in the same organism. Chromosomes have not been described in such cases nor are the metabolic conditions known, and the chief interest in such cases here is to emphasize the potentialities of each sex in the zygote; under normal conditions one set of potentialities predominates, the other being recessive. We do not know how this zygotic balance is so disturbed at times that both potentialities are expressed. Conceivably it may be based upon metabolic relations or again on other unknown conditions.

In postnatal differentiation in mammals objective results from experimentation have been less definite and striking than with the bird. This is due in large part to a lack of specific sex indicators in the ordinary laboratory animals on which the majority of investigations have been conducted. Steinach ('12, '13, '20) has employed body weight, body length, fat deposition, hair coat and the psychic nature, including sex inclinations, pugnacity, etc., in masculinized and feminized rats and guinea pigs as a criterion of sex.. Under careful analyses body weight and length, as I have shown by specific data (Moore '19, '21, '22), are subject to such wide individual

variations as to be wholly inadequate as sex indicators
excepting possibly where comparisons are capable of
being carried out on a large population of animals under
specific conditions. Hair coat and fat deposition are en-
tirely inadequate as qualitative expressions of sex. Psy-
chical disposition is a variable entity subject to wide
variation, and aside from the most general and outstand-
ing sex inclinations are to be avoided as indicators of
sexual conditions. Mammary glands are of considerable
importance in registering changes in hormonal effects.
Outstanding secondary sex characters are, therefore,
largely restricted to internal structures, such as seminal
vesicles, prostate, penis or its homologue the clitoris, vas
deferens, uterine tubes, etc.

Through castration experiments of Bouin and Ancel
('04), Steinach ('12), Sand ('19), Lipschutz ('19), the
writer and others we learn that the appearance and per-
sistence of most secondary sex characters of the male are
dependent upon the testis. General conditions can be
illustrated by the non-development of horns in some
castrated ruminants, the underdevelopment of the copu-
latory organs, prostate and seminal vesicles in practi-
cally any castrated male; body proportions in some forms
are altered through castration; hair distribution, size of
the larynx and other conditions may be altered in the
human after castration. Many examples of similar na-
ture could as well be given on the female side after
ovariotomy. But the fact of such modifications after
gonadectomy emphasizes also the fact that the supple-
mentary or hormonal influences are the chief factors in
the production of many of the most outstanding sex
differences. Lipschutz would believe that all sex differ-
entiation and even sex determination is dependent upon
hormones, thus postulating a primitive asexual form (or
soma) subject to primary differentiation in either direc-
tion. There are many facts, however, that would dis-
courage such an extreme view of the potency of hormones.

Experimental modification of sex differentiation in the
mammal by hormones have been attained almost entirely

by sex gland transplantation or removal. Slight and somewhat questionable indications have followed injections of extracts or emulsions of gonads, but on the whole this line of experimentation has been peculiarly barren.

In complete ovariotomized females Steinach, Lipschutz, Sand and the writer have described varying degrees of masculinization by testis grafts. These degrees, however, are based upon the visible sex indicators and herein lies the difficulty of application. Apparently, each hormone is sex specific and acts only upon those structures which it normally affects. Thus the uterus and mammae are unaffected by testis hormones. The only morphological effect detectable under such conditions in these females is on the homologue of the copulatory organ or clitoris. These authors as well as the writer have described in the guinea pig an increase in the size of this structure to such an extent that it resembles the male organ more than the female.

The psychical transformation, many times described, I personally consider a very questionable indicator. The fighting instinct and the disposition of a rat or guinea pig are such variable reactions that they appear entirely worthless. The sexual instincts, if interpreted broadly, may offer a somewhat more adequate criterion. There is considerable positive evidence that sexual instincts of a zygotically determined female may be developed under hormonal influences to a decided male type even to the point of faithful reproduction of a complicated and distinctive copulatory pattern; these reactions are in my experience totally foreign either to normal or ovariotomized females and are to be considered as having been conditioned by the introduced hormonal factor.

The reverse condition also holds true within limits— *i.e.*, a castrated male may undergo differentiation in a female direction under the influence of ovarian hormones. Chief among somatic modifications is an abnormal growth of the mammary glands in the male guinea pig, to the point of milk secretion, according to Steinach and Sand.

This appears to be the only morphological character capable of an unquestionable modification. Ovarian secretions do not affect the penis, prostate or seminal vesicles which in the absence of the testis remain underdeveloped. Latent homologous characters are, therefore, stimulated, but heterologous characters are unaffected.

Sex instincts are more confusing in experimental feminization than in masculinization, due to the fact that often the male impulses are acquired despite early castration and may persist for long periods of time after late testis removal. I have observed castrated male guinea pigs with ovarian grafts so effective as to influence mammae development to the size of normal females, that still retained the male psychic tendencies sufficiently persistent that one would judge them to be normal males from reactions to other animals; and this with an entire lack of any demonstrable female psychic tendencies.

The bisexual potentialities, therefore, though to a large extent latent for one sex under normal conditions, are capable of development to a certain extent when the proper hormones are introduced experimentally. In hermaphrodites the primary differentiation often expresses the bisexual potentialities by development of ovatestes or ovary and testis in the same animal, due to unbalanced zygotic factors; specific hormones from each sex gland may then further intensify the mixed sexual condition by causing the appearance of both types of secondary sex characteristics.

Experimentally, some secondary sex character intermixtures can be produced by the simultaneous grafting of both sex glands into a castrated individual, or again by transplantation of a gonad into a normal animal of the opposite sex. Thus a male guinea pig may be produced having a normally functioning genital tract and well-developed mammae; or a female with functional reproductive system may carry a testis graft having little or no visible demonstration of effects on secondary sex characters, but the graft itself undergoing the processes

of gamete production.   Sand in particular believes he
can recognize psychic bisexual expressions at the same
time.   Thus he reports a guinea pig acting at one moment
as a male and immediately thereafter as a female.   It is
possible, however, to become overenthusiastic in such in-
terpretations.

It should again be emphasized that, as in the bird, the
primary sex characters appear capable of striking experi-
mental modification during the course of their embryonic
development by hormonal agencies; but after their pri-
mary differentiation we have no evidence that secretions
from one gonad have any effect upon the other.   I have
shown that testes are perfectly normal when ovarian
grafts have been present in an active condition in the
male for months.   Similarly, females will reproduce nor-
mally when carrying large testis grafts before, during
and after pregnancy.   Sand first showed that ovaries may
be grafted into the substance of the testis and both glands
remain in a normal condition; this has been substantiated
both by myself and Lipschutz.

In review it may again be stated that to the best of our
knowledge sex determination occurs in the zygote; and
as Lillie ('23) has stated we may make the "assumption
that the zygotic sex-determining factors are also sex-
differentiating factors. . . . These factors are reinforced
very early by hormone production."   There is a great
deal of direct and indirect evidence that the sex-deter-
mining, as well as the sex-differentiating factors have a
quantitative as well as a qualitative aspect and that the
zygote contains the potentialities of both sexes; this is
beautifully illustrated in sex intergrades or hermaphro-
ditic forms.   The bisexual potentialities are clearly dem-
onstrated by grafting the opposite sex gland into an in-
dividual of the bird and to a somewhat lesser extent in
the mammal.   Experimentally, therefore, we can control
to some extent sex differentiation of a mammal or bird
and it is not beyond the possibilities that further knowl-
edge will provide us with instruments having much
greater possibilities than we can at present anticipate.

LITERATURE

Benoit, J.
>1923. *C. R. Soc. Biol.*, vol. 89, pp. 1326–1328.

Bouin and Ancel.
>1904. *Jour. de Physiol.*, vol. 6, p. 1039.

Crew, F. A. E.
>1923. *Proc. Roy. Soc. B.*, vol. 95, no. 667.

Domm, L. V.
>1924. *Proc. Soc. Exp. Biol. and Med.*, vol. 22, pp. 28–35.

Hance, R. T.
>1924. *Science*, vol. 59, no. 1532, pp. 424–425.

Lillie, F. R.
>1917a. *Jour. Exp. Zool.*, vol. 23, pp. 371–451.
>1917b. *Proc. Nat. Acad. Sci.*, vol. 3, pp. 464–470.
>1923. *Biol. Bull.*, vol. 44, pp. 47–78.

Lipschutz, A.
>1919. Berne.

Minoura, T.
>1921. *Jour. Exp. Zool.*, vol. 33, pp. 1–61.

Moore, C. R.
>1919. *Jour. Exp. Zool.*, vol. 28, pp. 137–160; 459–467.
>1921. *Jour. Exp. Zool.*, vol. 33, pp. 129–170; 365–390.
>1922. *Biol. Bull.*, vol. 43, pp. 285–312.

Painter, T. S.
>1922. *Jour. Exp. Zool.*, vol. 35, pp. 13–40.
>1923. *Jour. Exp. Zool.*, vol. 37, pp. 291–333.
>1924. *Jour. Exp. Zool.*, vol. 39, pp. 229–242; 433–460.

Pezard, Sand and Caridroit.
>1924. *C. R. Soc. Biol.*, vol. 90, p. 1459.
>1924. *C. R. de L'Acad. de Sci.*, vol. 178, p. 2011.

Riddle, Oscar.
>1917. *Science*, vol. 46, pp. 19–25.
>1924. AMER. NAT., vol. 58, p. 655.

Sand, Knud.
>1919. *Jour. of Physiol.*, vol. 53, p. 257.

Shiwago, P. J.
>1924. *Science*, vol. 60, pp. 45–46.

Steinach, E.
>1912. *Pflugger's Arch.*, vol. 144, pp. 71–106.
>1913. *Zentrl'bl f. Physiol.*, vol. 27.
>1920. *Arch. f. Ent'w-mech.*, vol. 46, pp. 12–28.

Stephens (figures published by Boring, *Science*, 1923, vol. 58).

Tandler and Keller.
>1911. *Deut. Tierarzt Wochenschrift.* Jahrg. 19 (cited by Lillie).

Zawadowsky, M.
>1922. Moscow (cited by Domm).

SHORTER ARTICLES AND DISCUSSION

THE EXISTENCE OF DOUBLE SPIRAL CHROMATIN BANDS AND A "BOUQUET" STAGE IN TRADESCANTIA PILOSA LEHM.

(Preliminary note)

THE difficulties which accompany the obtainment of adequate fixation of members of the genus *Tradescantia* for critical cell study, have been noted by several cytologists. During the past two years, the writer has experimented with a lengthy, graded series of fixing agents in an attempt to obtain good preparations of both somatic and meiotic cells of *Tradescantia pilosa* Lehm. In addition to the paraffin and celloidin methods, the smear method, recently described by Taylor,[1] was employed. These experiments reveal certain details of structure, which will be briefly mentioned here, prior to the publishing of a more detailed description.

In both somatic and meiotic phases, the chromatin is present in the form of a double spiral band. Indications of this structure have been observed in prophase, metaphase, anaphase and telophase chromosomes. It appears to be temporarily lost at the time of the division of the chromosomes to immediately reestablish itself in the daughter chromosomes.

The same spiral arrangement of chromatin has been observed in cells of *Rhoeo discolor* Hance.

There is no evidence in the heterotypic prophase of *Tradescantia* that synizesis is a natural occurrence. That, so often described aggregation of the chromatin material, frequently called synapsis by plant cytologists, does not appear in well fixed material. Instead, there appears a well defined "bouquet" stage, similar to that frequently figured and described by animal cytologists. A distinct attachment plate is visible at one side of the nucleus from which the spireme loops out into the nuclear cavity. It is important to note that in animal cells the "bouquet" stage associates itself with the presence of the centrosome.

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[1] Taylor, William Randolph, *Bot. Gaz.*, LXXVIII: 236–238. 1924.

A BALANCER IN LARVAE OF AMBLYSTOMA
TIGRINUM

THE absence of the balancers has been regarded as a distinguishing characteristic of the larvae of *A. tigrinum*. Balancers are found in other larvae, *Amblystoma opacum, jeffersonianum* and *punctatum*. Careful study of developing embryos of *A tigrinum* has shown that rudimentary balancers exist in some individuals of this species for a very brief period.

The eggs from which these embryos developed were laid in an aquarium by individuals secured last year in the axolotl stage from collectors in Wisconsin. These animals metamorphosed after being received in this laboratory and upon molting this spring displayed the striping characteristic of *Amblystoma tigrinum*.

The development of the eggs was observed closely and at that stage of development at which the first reflexes appeared (corresponding to Harrison's Stage 37)[1] a slight outpouching was noted in the region just posterior to the eye. The growth of this outpouching was studied. From its form and position in the mandibular region a relationship to the balancer found in other forms was surmised. The subsequent development and morphology showed beyond question that this was the case.

The balancers are present only during a very short period of development and are generally lost to external observation during the growth from stage 37 to stage 38, a period of six to eight hours at normal room temperature. Further studies on the internal development of this structure are in progress.

Aside from the importance of balancers in the classification of species, there have been several considerations of a theoretical nature advanced as to their function and significance. It was suggested by Clark ('80),[2] that the balancer was developed in order to keep the head and gills raised from the mud on which the animal normally rests during development and therefore more readily accessible to the supply of clearer water; and further, that these structures caused the elevation of the pericardial region above the substrate, thereby eliminating interference

[1] *Jour. Exp. Zool.*, Vol. 25.
[2] Studies from the Biological Laboratory of Johns Hopkins University, No. 11.

with the heart beat. Latta ('19)[3] seems to suggest that either of these explanations is plausible from his own observations.

These theoretical deductions concerning the significance and function of the balancer are not supported by the observations on *A. tigrinum*. There is no reason to suppose that larvae of this species do not require protection from the same adverse conditions as found in other species, yet the balancers are exceedingly rudimentary, non-functional and transitory.

Experimental studies in which the balancer bud, as the process extending behind the eye may be correctly termed, has been stimulated either by dividing the bud itself with a fine pair of scissors or by pricking the regions slightly anterior or slightly posterior to the bud region have given rather interesting results. The division of the balancer bud without the implantation of any inert material will not bring about the formation of a double balancer. The resorption of these structures, however, can be delayed by division of the balancer bud, and the period of their existence can be prolonged 24 to 36 hours. Stimulation of the region anterior to the bud also prolongs its existence. Stimulation of the materials posterior to the bud gave no results.

In the larvae of *A. punctatum* the balancer is broken off at the 14 mm stage. The proximal portion or base is later slowly resorbed. The transitory balancers of *A. tigrinum* are lost by resorption.

It must be concluded that at least in certain races of *Amblystoma tigrinum* a transitory balancer is found. This structure is never of functional importance in the form here studied. It is possible that other gradations in its development may be found in other races. The presence or absence of balancers is, therefore, not necessarily diagnostic of any one species of Amblystoma.

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[3] *Anatomical Record*, Vol. 17.